IDENTIFICATION OF INPUTS FOR A QUANTITATIVE MICROBIAL RISK ASSESSMENT OF *VIBRIO PARAHAEMOLYTICUS* IN CHESAPEAKE BAY AND WASHINGTON STATE

by
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ABSTRACT

Background / Introduction: Non-cholera *Vibrio* bacteria are a major cause of foodborne illness in the United States. Raw oyster consumption is increasingly implicated in gastroenteritis caused by pathogenic strains of *V. parahaemolyticus* (*Vp*). As oyster consumption expands and sea temperatures rise, *Vp* outbreaks may increase, posing major public health and food safety concerns. The US Food and Drug Administration (FDA) 2005 quantitative microbial risk assessment (QMRA) of *Vp* in raw oysters identified model uncertainties and information gaps that new research may address, underscoring the need for an updated QMRA. Further, the previous national risk model has not been adequately predictive for all US harvesting regions. Regionalization, using recent, localized studies, may improve precision of risk estimates by considering local harvest practices and variation of spatial and temporal factors.

Methods: A systematic review was conducted. Articles published from 2004 to 2018 were identified by a predefined search of scientific and government databases and Google Scholar. Two reviewers independently coded abstracts for inclusion/ exclusion and subsequent categorization using predefined criteria. Data was extracted and study quality and relevance were evaluated for studies categorized as post-harvest. Conclusions about efficacy of post-harvest practices and processes (PHPs) in achieving reductions in *Vp* abundance were synthesized using a weight of evidence approach.

Results: Of the 9,587 unique articles retrieved, 232 were included to inform dose-repose, harvest, post-harvest, and consumption components of the original QMRA framework. For post-harvest articles, data was extracted and evaluations of study quality and relevance conducted for 88 studies. 26 studies were found to be of both high quality and high relevance. High pressure and irradiation consistently emerged as the most effective PHPs in reducing *Vp.*

Conclusions: Overall, there is a larger body of literature on PHPs and their effectiveness in reducing *V. parahaemolyticus* growth than originally anticipated, but quality and relevance of the studies may limit the power and strength of regionalizing and updating the existing QMRA.

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CHAPTER 1. INTRODUCTION

Background

Public Health and Food Safety Concern

Dietary exposure to pathogenic *Vibrio parahaemolyticus* through seafood consumption represents an important public health and food safety concern. *V. parahaemolyticus* is a Gram-negative, halophilic, non-spore forming bacterium with an incubation period from 4 to 96 hours, with a median of 17 hours (Daniels et al., 2000). Infections of *V. parahaemolyticus* most commonly cause acute, self-limiting gastroenteritis, with clinical symptoms of diarrhea, abdominal cramps, nausea, vomiting, headache, fever and chills (FDA 2005, Baker-Austin et al. 2017).

V. parahaemolyticus is endemic to salty and brackish waters around the world (FDA 2012). Domestically, *V. parahaemolyticus* occurs naturally in the Chesapeake Bay and Puget Sound, two large estuaries on the U.S. East and West coasts. *V. parahaemolyticus* is taken up and concentrated by filter-feeding bivalves from ambient water (Yeung and Boor, 2004; Froelich et al. 2012). The main bivalves consumed by humans are oysters, mussels, clams, and scallops. Human exposure to *V. parahaemolyticus* can occur through consuming raw, undercooked or contaminated shellfish (Daniels et al. 2000). *V. parahaemolyticus* densities in oysters vary seasonally, and concentrations have been found to be positively correlated with water temperature, turbidity, and dissolved oxygen in the Chesapeake Bay (Parveen et al. 2008). Most strains of *V. parahaemolyticus* isolated from seawater and seafood are not pathogenic (Parveen et al. 2008).

A variety of virulence factors may promote pathogenicity of *V. parahaemolyticus*. These include the ecotoxins thermostable direct hemolysin (TDH) and thermostable direct-related hemolysin (TRH), expressed with the *tdh* and *trh* gene, respectively, as well as the type III secretion systems one and two (T3SS1 and T3SS2) (Letchumanan et al. 2014; Ceccarelli et al. 2013). The *toxR* gene stimulates expression of the *tdh* gene and may be found in pathogenic and non-pathogenic *V. parahaemolyticus* (Letchumanan et al. 2014). The *toxR* gene also encodes for

ToxR, a membrane regulatory protein and transcriptional activator that regulates expression of more than 60 genes, including genes for multiple virulence factors, in response to changes in the environment (Whitaker et al. 2010).The presence of different types of flagella for swimming and swarming, and capsule-producing ability, also promote the survival of virulent strains in the environment, and host colonization (Letchumanan et al. 2014). Over 95% of clinical isolates of *V. parahaemolyticus* were found to be positive for the *tdh* gene (Parveen et al. 2008).

Relative expression of different virulence factors has been shown to vary by region. For instance, clinical isolates from the Pacific Northwest during the 1997 to 1998 outbreaks contained the *trh* gene, and for the 1998 outbreak across 13 states, there was a rise in both *tdh* and *trh* genes in clinical isolates from infected persons (Letchumanan et al. 2014). Interestingly, significant concentrations of *tdh*+ and *trh*- isolated in environmental samples in the Pacific Northwest were not shown to be associated with illness (Paranjype et al. 2012). However, the *trh* gene is also found in *V. alginolyticus*, highlighting a need for additional verification methods to ensure that *trh* is indicative of *V. parahaemolyticus* and not the presence of *V. alginolyticus*.

Isolation and identification of infective strains of *V. parahaemolyticus* from shellfish associated with outbreaks have been problematic. The O3:K6 serotype caused outbreaks in India and Southeast Asia (Yeung and Boor 2004), and was responsible for the largest outbreak due to oyster consumption in the US, causing outbreaks in Texas, New York, New Jersey, and Connecticut (Daniels et al. 2000). However, it has not been associated with illness from raw oyster consumption in the US after 1999 (Su & Liu, 2006). Clinical samples were used to identify the strain, yet *V. parahaemolyticus* isolated from oysters at the sites did not identify as O3:K6 (CDC 1999). While *V. parahaemolyticus* falls under 13 O serotypes and 71 K serotypes, O3:K6 is known as the serovar responsible for the most outbreaks globally since 1996 (Osawa et al. 2002). Currently, over 20 serovars are known (Letchumanan et al. 2014). Further, differences between strains affect growth, survival and virulence (Yeung and Boor 2004). Many O3:K6 isolates have been found to contain the filamentous phage f237, positing an association between f237 and

widespread virulence of the O3:K6 serotype (Nasu et al. 2000). Within the phage, O3:K6 strains also have the gene *orf8*, which encodes for an adherence protein that renders O3:K6 more adhesive to host intestinal cells, thereby increasing the virulence of O3:K6 isolates (Letchumanan et al. 2014, Ceccarelli et al., 2013). Pandemic O3:K6 strains were also detected with the *toxRS* sequence, whereas non-pandemic O3:K6 strains did not (Letchumanan et al. 2014). The O4:K12 and O1:K56 serotypes have been linked to outbreaks from shellfish harvested in the Pacific Northwest and British Columbia in 1997 (Daniels et al. 2000).

Outbreaks of gastroenteritis associated with raw or undercooked shellfish consumption have occurred in the U.S since 1971, where it was first reported in Maryland (FDA 2005). All populations that consume oysters are considered at equal risk for self-limiting gastrointestinal illness (Yeung & Boor 2004). More susceptible subpopulations include individuals with immunosuppression, liver disease, or alcoholism (Daniels et al., 2000). Vulnerable subpopulations at greater risk of septicemia include those with cancer, liver, kidney or heart disease, antacid use, or recent gastric surgery. Septicemia can be fatal for these subpopulations and immunocompromised individuals (Yeung and Boor 2004). Less commonly, exposure of open wounds to water with high numbers of *V. parahaemolyticus* has resulted in wound infections and septicemia (FDA 2012, Daniels et al. 2000, Su and Liu 2007).

Harvest Practices

Oysters are harvested using a variety of practices that differ between different regions and introduce different opportunities for *V. parahaemolyticus* growth during harvest (Table 1). Oysters are often harvested intertidally in Washington state, due to tides that range by ten feet or more (Webster 2007). The Chesapeake Bay has much smaller tides, so intertidal harvest is not used (Webster 2007). During intertidal harvest, oysters are picked by hand and left in baskets, bags, nets or longlines during the low tide and are collected by boats during higher tides. The longer interim spent exposed to potentially warmer air temperatures before refrigeration may

allow for greater proliferation of *V. parahaemolyticus* during intertidal harvest than during dredging (FDA 2005). Studies have shown that mean densities of *V. parahaemolyticus* increased by a factor of four to eight from the initial exposure to after the maximum exposure in oysters (Nordstrom et al. 2004).

Table 1. Oyster harvesting practices by region.

Washington State[1, 3]	**Chesapeake Bay**[2, 3]
Aquaculture—Intertidal	**Aquaculture**
Bottom culture	Bottom culture (cages, dredging)
Stake culture	Rack and Bag culture
Longline culture	Floating culture
Floating culture	**Wild**
Suspended Nets	Dredging
Rack and Bag Culture	

[1] Source: Toba et al. 2002

[2] Source: NOAA 2018

[3] Source: Webster 2007

Post-Harvest Practices

Following harvest, *V. parahaemolyticus* multiplies quickly, even exponentially at room temperature and above (Yeung and Boor 2004). At room temperature, *V. parahaemolyticus* has a doubling time of 1.8 hours during the exponential growth phase and can increase 50-fold (1.7 log CFU/g) if left at room temperature for 10 hours and 790-fold (2.9 log CFU/g) at 24 hours (Gooch et al. 2002). Risk management strategies to control growth of *V. parahaemolyticus* include a variety of post-harvest practices (PHPs). Recommendations suggest immediate cooling of freshly harvested shellfish to prevent bacterial growth. Hygienic food handling processes such as maintaining low temperatures and avoiding cross-contamination are also critical risk reduction strategies (Yeung and Boor 2004). Heat, high-pressure, and irradiation treatments have been

shown to significantly reduce viable cell counts, reducing risk of infection, but more research is needed to assess the efficacy of these treatments on other strains of *V. parahaemolyticus* (Yeung and Boor 2004). The following are definitions of specific post-harvest practices (PHPs) studied in this text:

Depuration: A controlled process during which oysters are kept in clean seawater and can purge bacteria, sand or other contaminants from the gut. The efficacy of depuration in reducing bacterial counts is inconsistent across studies (WDOH 2014) and has been shown to be ineffective in reducing persistent bacteria such as *Vibrio spp.* in shellfish due to bacterial colonization in oysters' intestinal tracks (Su and Liu 2007).

Disinfectants: Chemical disinfectants, such as those containing chlorine and sodium chloride, are used to sanitize food contact surfaces in the food industry to prevent contamination between surfaces and food (Lin et al. 2013). Effectiveness of disinfectants to control cross contamination in the food service setting has been shown to vary by microorganism and may vary according to prior exposure to sublethal stresses, such as temperature shock or acidity (Lin et al. 2013).

Acid: During oyster post-harvest preparation and processing, *V. parahaemolyticus* may be exposed to acid interventions or acid stress conditions (Kalburge et al. 2014; Chiang et al. 2008a). Organic acids such as lactic acid are commonly used food preservatives and antimicrobial agents due to their ability to penetrate the cytoplasmic membrane (Lin 2004; Alakomi et al. 2000).

High hydrostatic pressure (HHP): HHP is applied to reduce enzymatic activity and bacterial abundance in foods (He et al. 2002). HHP may be applied to shucked or whole-shell oysters and can be used as a shucking process. *V. parahaemolyticus* has been shown to be highly susceptible to pressure treatments between 200 and 300 MPa (He et al. 2002). Though pathogenic and non-pathogenic strains vary in resistance to HHP, greater than 6 log reductions can be achieved even with more resistant strains and serotypes such as O3:K6 (FDA 2005).

Irradiation: Used to reduce pathogenic bacteria such as *V. parahaemolyticus* to non-detectable levels, as well as to kill spoilage bacteria (Thulipa et al. 2011). Gamma or X-ray irradiation is a commonly used method; irradiation using UV or LED are also emerging technologies. Irradiation has been shown to be highly effective across studies and can preserve oyster quality for consumers (Thulipa et al. 2011).

Relaying: Oysters are moved from one growing area to another growing area with lower levels of pathogens or pollution before harvest (Su and Liu 2007), often two weeks prior to distribution (WDOH 2014). The scarcity of unpolluted marine environments limit potential for use of the relaying technique (Su and Liu 2007).

Re-submersion: Another common antifouling practice involves exposing oysters to ambient air conditions during dry storage, following re-submersion for approximately 7 days at the same site (Kinsey et al. 2015). Effectiveness of re-submersion in reducing *V. parahaemolyticus* has been limited. Re-submersion is practiced differently for intertidally harvested oysters. Exposure to ambient air temperature and radiant heat during intertidal exposure promotes growth of *V. parahaemolyticus* during intertidal harvest (Jones et al. 2016). With tidal re-submersion, oysters are submersed with the incoming tide and may purge *Vibrio spp.* (Jones et al. 2016).

Cold storage: Storage at low temperatures for days to weeks is used to preserve oysters and limit growth. Oysters may be stored in refrigeration units, freezer units, or ice. Cold storage is commonly used after application of other PHPs.

Icing: Icing is used to directly chill oysters, either immediately on-board after harvest (Thomas 2016) or at dockside after harvest (Melody et al. 2008a). Icing may be applied for several hours prior to cold storage.

Temperature shock or stress: Environmental stresses such as heat, cold, or freeze-thaw are used to reduce bacterial abundance and virulence. During freeze-thaw, samples are kept frozen and intermittently thawed at room temperature and re-frozen (Hasegawa et al. 2013).

Alternatively, oysters may be subjected to cold shock during rapid freezing and then kept in cold or frozen storage for weeks at a time (FDA 2005). During heat shock, oysters are exposed to elevated temperatures for a period of time.

High hydrostatic pressure (HHP) and irradiation applied alone or in combination with thermal processes can effectively reduce and eliminate foodborne pathogens such as *V. parahaemolyticus* in oysters (Su and Liu 2007).

Current Regulatory Guidelines

To limit growth of *V. parahaemolyticus* while oysters are exposed to higher temperatures, the National Shellfish Sanitation Program Guide for the Control of Molluscan Shellfish requires that shellfish need to cool to internal temperatures of 10°C within 12 h, 18 h, 24 h, and 36 h of harvest when the mean maximum air temperature of the month of harvest is > 27°C, 15 – 27°C, 10 – 15°C, and < 10°C, respectively, when harvested for raw consumption (NSSP 2017). During periods when the risk of illness with *V. parahaemolyticus* is reasonably likely to occur, the original dealer must cool oysters to internal temperatures of 10°C within 10 h or less (NSSP 2017).

For dealers that elect to use a PHP to reduce levels of *V. parahaemolyticus* in oysters and seek to label oysters at retail as processed by a PHP, the dealer must use a process that has been validated to reduce the level of *V. parahaemolyticus* to non-detectable levels below the limit of detection (LOD, < 30 MPN/g) and achieve a minimum 3.52 log reduction (NSSP 2017). Given existing guidance, 3.52 was selected as the standard log reduction a PHP must consistently achieve to be recommended as an effective mitigation strategy for this thesis.

Quantitative Microbial Risk Assessments: FDA 2005, WDOH 2014

The United States Food and Drug Administration (FDA) conducted a quantitative microbial risk assessment (QMRA) in 2005 to determine the factors that influence the risk of infection and

illness from consumption of pathogenic *V. parahaemolyticus* in raw oysters, and to examine the public health impact of control measures. The FDA QMRA framework was comprised of four modules, dose-response, harvest, post-harvest, and consumption.

The FDA QMRA was conducted in response to four major outbreaks that occurred in the U.S., causing over 700 cases of foodborne illness in 1997 and 1998 (FDA 2005). The outbreaks highlighted the need to assess the efficacy of risk management guidance in place at that time, including the FDA guidance for maximum acceptable number of *V. parahaemolyticus* per gram of shellfish, and other management strategies. The FDA QMRA used national models to predict mean risk of illness per serving and mean annual number of illnesses for six oyster harvesting regions and four seasons, including the Mid-Atlantic and Pacific Northwest, divided into intertidal and dredged harvesting regions. The mean risk of illness per serving of raw oysters was 1.3×10^{-4} for the Mid-Atlantic (total across seasons); 1.1×10^{-5} for the Pacific Northwest – dredged (total), and 1.5×10^{-4} for the Pacific Northwest – intertidal (total). The mean annual number of illness in the U.S. each year for oysters harvested from the Mid-Atlantic was 15; for oysters harvested using dredging in the Pacific Northwest, 4; and for oysters harvested intertidally in the Pacific Northwest, 2,826 (FDA 2005). Risk and number of illnesses varied by season, with higher predicted risk and number of illnesses during the spring and summer (FDA 2005).

The FDA QMRA also estimated reductions in risk associated with post-harvest treatments and found that treatments that reduced *V. parahaemolyticus* by 2 to 4.5 logs were effective for all seasons and regions, with greater effects observed with higher baseline risk (FDA 2005). Treatments that met or exceeded 4.5 log reductions reduced the probability of illness such that few cases would be identified through epidemiological surveillance (FDA 2005). Freezing was found to achieve 2 log reductions, while mild heat, ultra-high pressure or irradiation achieved 4.5 log reductions. Some outbreak strains exhibit higher resistance to post-harvest practices than endemic pathogenic strains, and incidence of pathogenic strains appeared to be greater in the Pacific Northwest (FDA 2005).

Given limitations in information available at the time, the FDA QMRA used assumptions to account for incomplete data. Uncertainties in the models were due to insufficient data. Future research needs highlighted in the FDA QMRA included: data on the incidence and frequency of relative abundance to total of pathogenic *V. parahaemolyticus* in water and shellfish, impact of overnight submersion of intertidally harvested oysters; growth rate at temperatures other than 26°C; impact of post-harvest handling and processing, survey of the oyster retail market and consumption information for the different regions, improved dose-response data, particularly on the difference in virulence between strains and virulence factors other than TDH, improved state surveillance systems and data, and post retail consumer handling of raw oysters (FDA 2005).

The FDA QMRA used national-level models or regional data assumed to represent conditions and trends in other regions. There may be limitations to national-level models that reduce their predictive ability for specific regions. Regionalization of the risk assessment, using recent, localized studies, would likely improve predictions and thus improve certainty in conclusions developed for a region. The Washington Department of Health developed a risk assessment specific to Washington State in 2014, which aimed to better characterize exposure and risk of illness resulting from intertidal harvest. The conceptual model developed in the FDA QMRA was used for the WDOH report, but with greater emphasis on intertidal exposure and substrate effects; a unified growth model; and inputs where users may submit static values. Serving size was estimated from WDOH illness logs collected from 2011-2013, where number of oysters consumed was self-reported by patients. The growth rate was developed with data from Yang et al. (2009), which measured growth on salmon tissue at five temperatures, ranging from 16 to 35°C. The data were re-fit to three-phase linear models without a lag phase, as recommended in FDA 2005. To determine cool-down rate, data from Schwarz 1999 was used and largely supported a monotonic relationship between time and cool-down temperature (WDOH 2014). WDOH 2014 used the beta-Poisson dose-response model developed by FDA 2005. As assumed in the FDA QMRA, the WDOH risk assessment also assumed that pathogenicity is

represented by *tdh* alone, and percentage pathogenicity is temperature independent. WDOH also assumed that serotype did not affect pathogenicity, and pathogenic and non-pathogenic bacteria incorporate into oyster tissue only influenced by water temperature (WDOH 2014).

The WDOH 2014 risk assessment did not incorporate re-submergence or other PHPs into the model, assuming that re-submergence was not used after harvest or during storage. Though re-submergence and depuration are commonly used in Washington, the report cited the variety of re-submergence strategies used, as well as the lack of consistent data on the efficacy of re-submergence or depuration in reducing *V. parahaemolyticus* abundance, as reasons for its omission. WDOH 2014 represented the first effort to regionalize risk models for *V. parahaemolyticus* in raw oysters to Washington state. The report aimed to serve as a starting point for future work to refine the models with improved and expanded data.

Specific Aims

The FDA 2005 and the WDOH 2014 risk assessments identified uncertainties and information gaps that new research could address, underscoring the need for an updated QMRA that incorporates findings from recent studies. The goal of this thesis is to identify novel research that can support the development of a regionalized QMRA for the risk of illness from *V. parahaemolyticus* due to consumption of oysters harvested in the Chesapeake Bay and Washington state. Further, this thesis aims to support more efficient expenditure of resources and a better use of post-harvest practices and controls, to reduce the burden of seafood-born *V. parahaemolyticus* infections. This work aims to fill information gaps, challenge assumptions and address uncertainties from previous risks assessments through the following specific aims:

Aim 1. Conduct a systematic review to identify studies that assess dose-response relationships, factors influencing population dynamics of *V. parahaemolyticus* from harvest to consumption, and oyster consumption behaviors.

1A. Conduct searches using predefined, controlled vocabulary and keyword terms in scientific and government databases to identify recent literature pertaining to *V. parahaemolyticus* dose-response relationships, population dynamics, and shellfish consumption behaviors.

1B. Identify relevant literature through title and abstract screening according to inclusion criteria and categorize by topics(s) and region. Studies were screened out of further review if they met categories for exclusion using exclusion criteria.

<u>Aim 2.</u> Evaluate studies identified for the post-harvest module based on study quality and relevance.

2A. Evaluate study quality with predefined criteria including study design, execution, analysis, reporting, and data strength. Perform risk of bias assessment and evaluate *V. parahaemolyticus* enumeration methodology.

2B. Evaluate study relevance based on region, testing of FDA 2005 model assumptions, and data strength to support a structural effect in the exposure assessment model and parameter impact in post-harvest models.

<u>Aim 3:</u> Synthesize findings using a weight of evidence approach. Provide recommendations for studies to be included in the post-harvest module and propose changes and regionalized inputs to the post-harvest module for a future QMRA.

3A. Summarize evidence and provide recommendations for modifications to the framework and models for the post-harvest module for the QMRA of *V. parahaemolyticus* in raw oysters, and identify potential new, regionalized inputs for future risk assessments.

3C. Propose guidelines to evaluate studies included in the dose-response, harvest, and consumption modules.

Aim 2 will evaluate studies identified for review in Aim 1 using structured criteria assessing the quality of the study methodology and design, and relevance to the QMRA. The

evaluation of data in Aim 2A will inform the synthesis of findings and form conclusions on the current state of the evidence, using a weight of evidence approach for Aim 3.

Impact

Implementation of these three aims will result in recommendations regarding the selection of studies for the dose-response, harvest, post-harvest, and consumption modules, as well as potential modifications to the post-harvest module for a future risk assessment of *V. parahaemolyticus* in raw oysters harvested from Chesapeake Bay and Washington. Identification of recent data to inform the input values and distributions of variability and uncertainty promote more up-to-date and informed parameters, which in turn improve the accuracy and precision of QMRA models. Additionally, data may also suggest structural changes to the QMRA models that result in improved predictions of risk and an overall improved risk assessment based on the best available scientific evidence. Use of QMRA model findings that better capture the effectiveness of PHP interventions and implications for disease risk can inform regulatory guidance on conditions needed to achieve the recommended log reductions for safe consumption. This thesis aims to inform public health policy on effective PHP interventions to reduce the burden of disease from consuming *V. parahaemolyticus* in raw oysters harvested from Chesapeake Bay and Washington state.

Significance

V. parahaemolyticus is a common cause of foodborne illness throughout Asia and seafood-associated illness worldwide. Chesapeake Bay and Washington state harvesting waters represent critical U.S. economic estuaries for bivalve harvesting, primarily through aquaculture (NOAA 2015). Washington state leads the nation in production of farmed bivalves (NOAA 2015). The main bivalves consumed raw or undercooked in the U.S. are oysters, mussels, clams, and scallops. Shellfish landings revenue made up 65% of total revenue (total $558 million) for the Pacific Region (NOAA 2015). For the Mid-Atlantic region, shellfish landings revenue accounted for 77% of total revenue (total $512 million). Across the United States, shellfish revenues totaled

$2,826 million, and 1,123 million pounds of landings were caught (NOAA 2015). Non-cholera *Vibrio* bacteria are estimated to cause 80,000 illnesses, 500 hospitalizations and 100 deaths in the U.S. each year (Scallan et al. 2011). Reporting shows that incidence of all major bacterial foodborne pathogens is declining in the United States, with the exception of *Vibrio spp.* and specifically *V. parahaemolyticus* incidence increasing in recent years (Baker-Austin et al. 2017, Scallan et al., 2011). With increasing consumption of oysters and warming sea temperatures due to climate change, *V. parahaemolyticus* outbreaks have the potential to increase, posing major public health and food safety concerns.

Climate change is already affecting the relationship between environmental parameters and *V. parahaemolyticus* populations, with potential implications for disease burden. Due to the positive correlation between temperature and bacterial counts of *Vibrio spp.*, climate change-induced warmer temperatures can foster an expanding geographic range and accelerating growth rate of *V. parahaemolyticus* in the estuarine environment (Baker-Austin et al. 2017). Anthropogenic climate change has caused rapidly warming temperatures globally, and a majority of that warming (over 90%) is stored in oceans (Cheng et al. 2017) connected to estuaries. In fact, Baker-Austin et. al (2017) report that pathogenic *Vibrio spp.* epitomize an "important and tangible barometer" of climate change in marine systems. Temperate regions such as Northern Europe have experienced an increase in human infections and outbreaks with *Vibrio spp.* connected to heat waves and other extreme weather events that will become increasingly common under current climate forecasts (Baker-Austin et al. 2017). As a mild to moderate halophile (optimal salinity range from 1.5-3.0% NaCl) (WHO 2011), *V. parahaemolyticus* growth increases when salinity increases from 1 to 3% (Whitaker et al. 2010). Climate change induced sea level rise is projected to increase estuarine salinity (Hong and Shen 2012), potentially promoting *V. parahaemolyticus* growth. Of note, the relationship between environmental salinity and *V. parahaemolyticus* abundance was determined to be a function of temperature and turbidity. Low

temperature or turbidity has been found to negate the positive effect of high salinity (Davis et al. 2017).

This thesis falls within a broader project aiming to assess the spatial and temporal variation of *V. parahaemolyticus* and associated health risks within the tidal waters of the Chesapeake Bay and Washington state. Oysters harvested from Chesapeake Bay and Washington state have caused outbreaks of *V. parahaemolyticus* infections over the last several decades (Su and Liu 2007). In fact, in 2018 there were a high number of confirmed *Vibrio* illnesses from people who consumed raw shellfish across Washington state during the warmer months (Dawson 2018). This trend is particularly concerning given the prior success of the Washington State Board of Health revised *V. parahaemolyticus* Control Plan Rule, adopted in 2015 (WDOH 2015).

Research on the spatial (e.g. geographic region of oyster harvest) and temporal (e.g. time from harvest to cold storage) variation of risk factors may improve predictions for abundance of *V. parahaemolyticus* in oysters and promote more effective and efficient control measures. Regionalization better characterizes exposures specific to highly different regions. For example, the intertidal harvest method is widely used in Washington state due to the large tidal range, but not in the Chesapeake Bay, which tends to have much smaller tides (Webster 2007). The length of time oysters are left out of water and exposed to high temperatures before refrigeration varies based on season and intertidal method. Additionally, regional trends in the presence and abundance of certain virulence factors have been observed (DePaola et al. 2003). Emerging research in this area has the potential to inform variability in exposure across and within different harvesting and post-harvest methods, thus improving risk characterization.

Innovation

Recently published research can account for limitations and provide major updates to the FDA QMRA, with the potential to substantially alter the existing framework. This project will compare the state of evidence synthesized from recent literature, to findings used in the FDA QMRA. Further, the validity of the assumptions for the dose-response model, population

dynamics (harvest and post-harvest models) and consumption behaviors (consumption model) may have been challenged by research released since that gathered for the 2005 risk assessment.

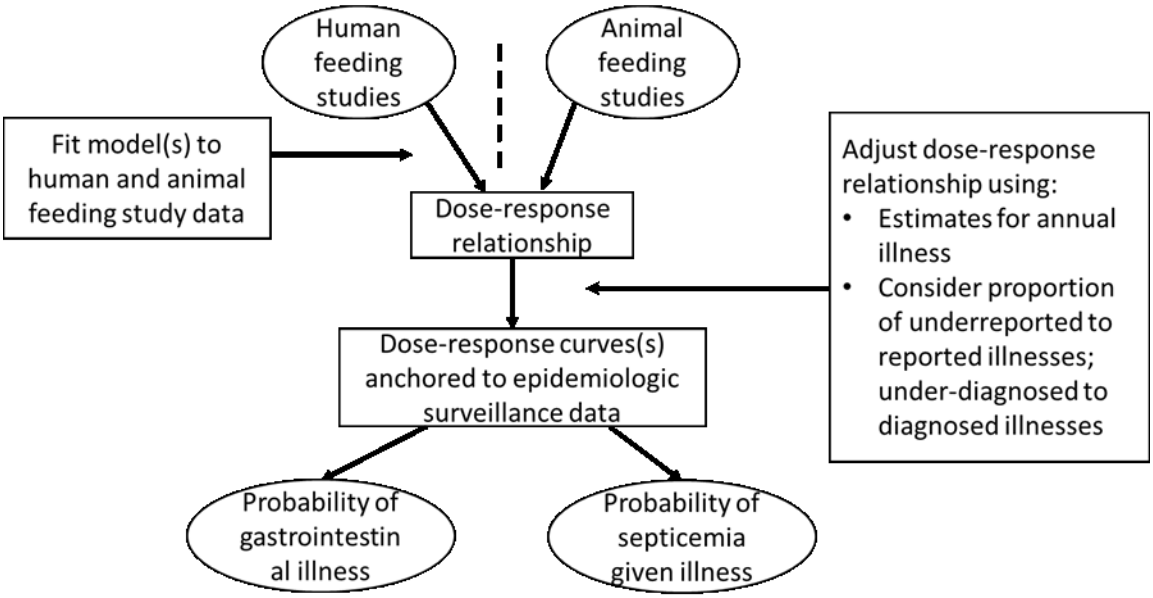
Additionally, this QMRA aims to apply more precision to forecasts through regionalization. While the FDA QMRA used nationally representative models based on data collected from multiple states and applied them to specific oyster harvesting regions, the proposed QMRA will be regionally specific to two major oyster harvesting economies in the U.S.: Washington state and the Chesapeake Bay. The new scope necessitates new criteria relevant to the purposes of this research. While the WDOH QMRA is specific to Washington State, it employed conceptual models based on the FDA QMRA and highlighted information gaps for which extensive research published since 2013 may challenge or address. Thus, this approach will lay the groundwork to provide an improved estimation of risk of illness from exposure to a foodborne pathogen from harvest to consumption.

To model the dose-response relationship, the FDA QMRA used human clinical feeding studies with pathogenic *V. parahaemolyticus.* The human feeding trial data (Aiso and Fujiwara, 1963; Sanyal and Sen, 1974; Takikawa, 1958) were pooled and fit with a Beta-Poisson distribution. 9 of the 20 total healthy subjects developed gastroenteritis symptoms; no symptoms occurred at low doses but 50 to 100% of subjects developed gastroenteritis at higher doses. Protein-rich meals (i.e. oysters) increase stomach acidity, so the infective dose of *V. parahaemolyticus* is expected to increase with consumption of oysters, as would be expected in the general population (FDA 2005). To account for the disparity, the authors anchored the dose-response model at the CDC's estimated average annual illness burden (2,800 cases) and estimated the number of servings consumed to form an adjustment factor of 27. After adjustment, the Beta-Poisson model dose associated with 50% probability of illness (ID_{50}) was $2.8x10^6$ organisms/serving (FDA 2005). Model choice will be re-examined, considering human and animal feeding studies, to understand if a better model(s) improves forecasting capabilities and provides a better fit at realistic, real-world exposure doses.

While the FDA QMRA excluded animal data from its dose-response model, there is potential utility in using animal studies to extrapolate to human dose-response estimates; thus, animal studies of *V. parahaemolyticus* will be included in the review. There is precedent in the literature for using animal models, specifically engrafted mice models, for assessing human response to bacterial pathogens. Song et al. (2010) found that engrafted mice displayed human innate and adaptive immune response to *Salmonella typhi*, also a gram-negative bacterium. The Song et al. 2010 study has been widely cited and indicates the potential for using mouse models and humanized mice for *in vivo* studies (Shultz et al. 2012).

Incorporation of animal animals to the dose-response relationship is depicted in Figure 1, adapted from the FDA (2005) dose-response assessment structure.

Figure 1. Structure of Dose-Response assessment model. Adapted from FDA 2005 Figure III-1.



The exposure assessment component of the risk framework defines the likelihood (based on frequency and probable level) of exposure to *V. parahaemolyticus* from consuming raw oysters (FDA 2005).

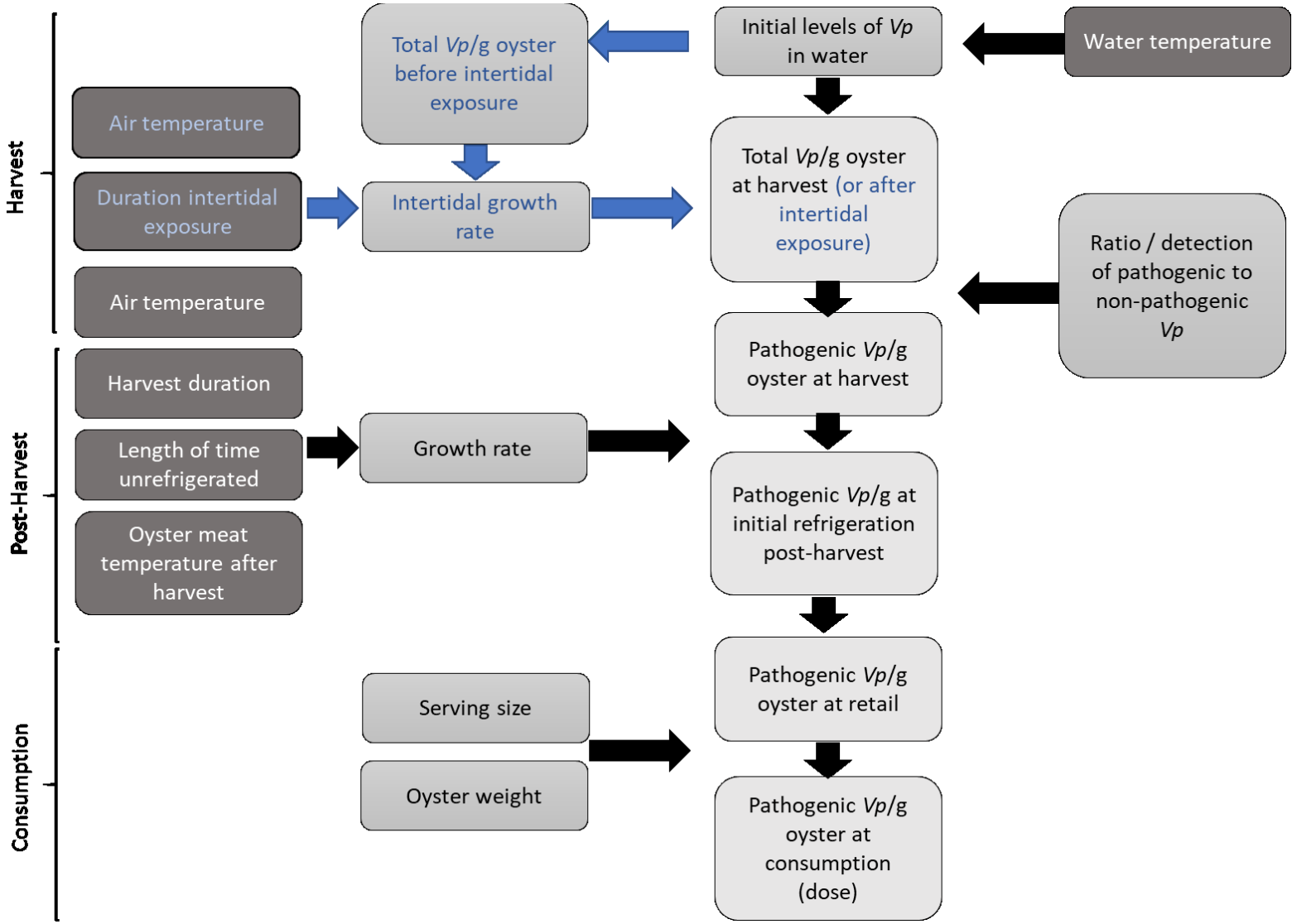
To conduct the exposure assessment, the FDA QMRA modeled the chain of events from oyster harvest to post-harvest handling and processing, to consumption (Figure 2). WDOH (2014) based its exposure assessment structure on the FDA (2005) structure, with added inputs to support growth during intertidal exposure. Integration of both exposure assessment structures is depicted in Figure 2. The harvest module assumed that oysters comprising a serving at consumption are harvested at the same time / location; pathogenesis is indicated by the presence of the virulence factor thermostable direct hemolysin (TDH) with the *tdh* gene; and the relationship between pathogenic and total *V. parahaemolyticus* is temperature independent (FDA 2005). Assumptions used in the WDOH 2014 report were based on FDA 2005 assumptions. WDOH 2014 used data from Yang et al. (2009) to construct the growth model, based on a pathogenic strain of *V. parahaemolyticus*, serotype O3:K6.

These assumptions may not be substantiated by current evidence. Recent literature suggests that pathogenicity of *V. parahaemolyticus* increases with temperature (Baker-Austin et al. 2017), highlighting the need to update the assumptions. Furthermore, additional virulence factors are known to indicate pathogenic strains of *V. parahaemolyticus*, such as thermostable-related hemolysin (TRH) expressed with the *trh* gene, the type III secretion systems T3SS1 and T3SS2, and biofilm formation (Letchumanan et al. 2014; Ceccarelli et al. 2013; Zhang and Orth 2013). Recent outbreaks of *V. parahaemolyticus* illness caused by strains that lack *tdh* and/ or *trh* genes highlight the importance of considering other virulence factors (Mahoney et al. 2010). Studies using more modern methods should be able to more precisely capture pathogenic *V. parahaemolyticus* abundance. Unique environmental associations of pathogenic strains have also been suggested, potentially due to their ability to enter a viable but nonculturable (VBNC) state in cold conditions. Though estuarine environments in northern New England tend to have relatively

cool water temperatures, they are a known niche for pathogenic *Vibrio spp*. and pathogenic *V. parahaemolyticus* (Mahoney et al. 2010).

The literature will be evaluated given this information and the extent to which it considers additional virulence factors and modern detection methods, so that the synthesis reflects an updated and current state of the evidence.

Figure 2. Schematic representation of Exposure Assessment for Vp in raw oysters. Adapted from FDA 2005 Figure IV-1; WDOH 2014 Figure 1. Blue text indicates inputs for WA state model using intertidal harvest. Dark grey boxes indicate input values; medium grey boxes indicate parameter values; and light grey boxes indicate abundance of Vp at stages of the supply chain.



Recent research tracking *V. parahaemolyticus* levels during stages of post-harvest handling and processing may address information gaps and provide input information for the FDA QMRA. Model inputs that determined the Growth Rate Model are: harvest duration, growth rate of *V. parahaemolyticus* as a function of air temperature, oyster meat temperature after harvest, and length of time unrefrigerated (FDA 2005). To account for a lack of information to inform those inputs, the authors relied on summary experimental data as raw data were not available; discrete approximation to model growth during cooldown; surrogate measures, such as total *V. parahaemolyticus* as a surrogate for pathogenic *V. parahaemolyticus* and air temperature as a surrogate for oyster meat temperature; and inference of the infective dose when *V. parahaemolyticus* is consumed with oysters, as doses were administered with antacids in most cases in the human feeding trials (FDA 2005). As harvesting methods are specific to the oyster harvesting region, modelling how *V. parahaemolyticus* abundance in oysters increased during intertidal or other specific harvest practices can reduce uncertainty and increase understanding of the variability in baseline abundance expected before application of a post-harvest practice (PHP). Incorporation of regional PHPs and their temporal relationship with growth of *V. parahaemolyticus* will allow for a more complete and nuanced understanding of exposure for consumers of oysters harvested from Chesapeake Bay and Washington.

Findings from studies that inform the Harvest, Post-Harvest, and Consumption Modules will be evaluated based on quality of data and analysis, regional relevance, and ability to address uncertainties of the existing QMRA where possible. Studies will also be evaluated on use of virulence factor detection methods, and consideration of virulence traits associated with pathogenicity. New laboratory detection methods for *V. parahaemolyticus* and their relative capabilities for identifying virulence potential may enhance the predictive capabilities of the risk assessment.

For the Post-Harvest Model, studies will be evaluated for their coverage of factors associated with post-harvest handling and processing of oysters. Studies will be evaluated on their

ability to inform growth, survival, or reduction of *V. parahaemolyticus* populations under a variety of experimental conditions (i.e. range of temperatures, duration times, and other treatment levels used) and which practices impact abundance (growth) of *V. parahaemolyticus* from the time of harvest to refrigeration. Finally, regulations regarding post-harvest practices will be used to assess the effectiveness of PHPs as indicated in study findings.

Following data extraction and study evaluation in Aims 2A and 2B, models, inputs, and parameters from the reviewed literature will be reported in tables to summarize the body of evidence. Data quality, consistency, and magnitude of effect will be considered. Conclusions will be drawn on the body of evidence for each PHP using a weight of evidence approach.

The following definitions will be used to describe how existing research may impact the existing FDA QMRA:

Framework: The basic structure of the QMRA, from problem formulation to risk management (Figure 1); the framework is comprised of four modules: dose-response, harvest, post-harvest, and consumption. The modules are comprised of models that, in turn, demonstrate key assumptions.

Model: The form of the process that generates data for dose-response relationships, population dynamics, and consumption behaviors. The population dynamics model is further divided into the harvest and post-harvest models. The models are composed of parameters.

Parameter: Statistical characteristics of inputs that are used to inform the models and quantify the relationship of interest.

CHAPTER 2. SYSTEMATIC REVIEW: IDENTIFICATION, DATA EXTRACTION OF STUDIES

INTRODUCTION

Aim 1. Conduct a systematic review to identify studies that assess dose-response relationships, factors influencing population dynamics of *V. parahaemolyticus* from harvest to consumption, and oyster consumption behaviors.

Systematic review methods were used to identify recent studies for inclusion in a future QMRA to inform all modules: dose-response, harvest, post-harvest, and consumption. Systematic review methods were selected as they pose multiple advantages over traditional narrative review methods. During systematic review, comprehensive and systematic literature searches identify findings from all database resources available, and diverse databases and search engines are often used to capture more available resources (Pae 2015). Objective and pre-defined inclusion criteria used in systematic review minimizes selection bias and avoids subjective selection bias (Pae 2015). Data extraction is protocol-based, and foundational to data synthesis. Data quality is evaluated based on guidance from multiple other sources, and interpretation of findings is based on data that were included and evaluated. Narrative reviews are more subject to the author's interpretation and subjective conclusions (Pae 2015).

Since 2004, when most literature gathering was assumed to have been completed for the FDA QMRA, thousands of primary research papers have been published on *Vibrio spp.*, seafood-borne illness, or at the intersection of both topic areas. Numerous review papers have also been published. Robust and systematic methods were needed to identify all available papers that were relevant to the scope of the project. The objective of this systematic review was to determine whether evidence published since 2004 and through 2018 supported or challenged the conclusions of the existing QMRA.

Application of formal systematic review methods to food safety risk assessments is an emerging field. Systematic review to support food safety risk assessment presents novel and

important opportunities for the rigorous, pre-defined and standardized methods of systematic review to be applied to guide in the identification and assessment of relevant research to support food safety decision making (EFSA 2010).

METHODS

Overview

Literature was identified for review using a defined search strategy for scientific databases, Google Scholar, and a targeted government report search. The search was conducted in summer 2018, and the citation information, titles and abstracts for all studies identified by the search strategy were downloaded and catalogued. The PICO (population, exposure or intervention, comparator, outcome) framework (Table 2) was used to determine inclusion-exclusion criteria (Tables 3, 4) subjected to adjudicate titles and abstracts. Studies were screened out of further review if they met categories for exclusion using exclusion criteria (Table 5). Selected studies were subsequently categorized according to the appropriate QMRA component, dose-response, harvest, post-harvest, or consumption, with information extracted systematically according to pre-determined parameters.

Data and information were extracted from studies that met criteria for inclusion for full-text review based on a pre-determined framework to inform the dose-response assessment, exposure assessment on harvest and post-harvest *V. parahaemolyticus* population dynamics, and oyster consumption behaviors.

Identification

Search Strategy

A search strategy was developed for the dose-response, harvest, and post-harvest modules; a separate search strategy was developed for the consumption module given the types of sources that were expected to inform the consumption module. An Informationist (Lori Rosman) from Welch Medical Library helped develop the search strategies and tailor it to each database, such as

using Mesh terms for PubMed, Index terms for Embase, and Keywords on Scopus. Searches were conducted in July and August 2018. All searches were date limited from 2004 to 2018, without any language restriction. Results from PubMed, NAL Agricola, Science.gov, and Google Scholar were first exported into RefWorks and later imported into Covidence. Scopus and Embase search results were downloaded in .ris file format and directly exported into Covidence. All search strategy results from all databases were de-duplicated in Covidence.

The search strategy for the harvest and post-harvest modules were divided into three concepts: 1) bacteria name; 2) vibrio infection and illness; 3) vibrio and harvest. The first concept included bacterial name, "Vibrio parahaemolyticus," "v. parahaemolyticus," and a common alternative spelling, "Vibrio parahemolyticus" The second concept included combinations of "Vibrio" with "Infections" or "Illness*," as well as "Vibriosis" and "Vibrioses." The third concept included "Vibrio*" and "harvest." Depending on the database, some specifications for proximity of "vibrio parahaemolyticus" with "illness*" or "infect*," as determined by the Informationist.

The search strategy for the consumption module was divided into two concepts, 1) raw oysters or shellfish; and 2) source type or study topic. Terms used for concept one included "oyster*" or "raw shellfish." Terms used for concept two included "food quality," "feeding behavior," "diet surveys," "questionnaire," "consume*," and "consumption." The search strategy for Google Scholar was combined into one concept as "raw oyster consumption behavior*." The complete search strategies are available in Appendix Tables A1 – A6.

Screening

Title and abstract screening were conducted to identify potentially relevant studies that may assess dose-response relationships, factors influencing population dynamics of *V. parahaemolyticus* along the supply chain from harvest to consumption, and oyster consumption behaviors**.** Titles and abstracts underwent title and abstract screening using Covidence. Studies determined to be relevant to the scope of the project met at least one of the inclusion criteria, and

were published between 2004 and 2018. Inclusion criteria were developed according to the PICO statement (Table 2) and with input from a subject matter expert (Dr. Angelo DePaola). Studies that met at least one of the inclusion criteria, in addition to date, were considered potentially relevant (Table 3). For studies screened to be potentially relevant, citation information and abstracts were downloaded into Excel.

Inclusion and Exclusion Criteria

The PICO statement (Table 2) was used to form the inclusion and exclusion criteria (Table 3) by specifying questions asked in the dose-response, harvest, post-harvest, and consumption modules according to the basic question structures for food safety risk assessments (EFSA 2010). Basic question structures fall under three categories: 1) effects of an intervention or exposure, where the population, intervention or exposure, comparator, and outcome need to be specified; 2) test accuracy (sensitivity and specificity); and 3) descriptive questions of populations, such as about prevalence, consumption and incidence of the specified outcome in the population of interest (EFSA 2010). As all the question structures may be answered through a systematic review of primary research, systematic review methods were used (EFSA 2010).

Dose-Response: Dose-response questions study the relationship between a factor (exposure to *V. parahaemolyticus*) and its effects on human populations (gastrointestinal illness) exposed to different doses (the comparator). Dose-response questions may fall under the following basic question structures for food safety questions: 1) questions about test accuracy; and 2) descriptive questions.

1. Questions about test accuracy involve diagnostic test accuracy of methods used.
    a. Population (P): Humans or other animals (such as salmon) to which *V. parahaemolyticus* is administrated. For human feeding trials, the food matrix (such as, was the dose administered with an antacid, with oyster meat, or alone?) is properly defined for the diagnostic test.

b. Index test (I): sensitivity and specificity of methods used to detect or measure incidence of the target condition.

c. Target condition (T): Gastrointestinal illness in humans or a relevant endpoint in animals or cells.

2. Descriptive questions examine prevalence and incidence of gastrointestinal illness caused by consumption of raw oysters.

   a. Populations (P): who consume oysters harvested in the Chesapeake Bay and Washington state oyster harvesting regions. Populations of interest are those who are more vulnerable or susceptible (are older or immunocompromised, have liver disease or alcoholism).

   b. Comparator (C): dose levels administered to determine the dose-response relationship in controlled studies or trials. Different food matrices examined (such as, dose administered with oyster meat, antacid, or alone).

   c. Outcome (O): Prevalence or incidence of gastrointestinal illness caused by *V. parahaemolyticus* is measured in the population specified above. For occurrence in raw oysters, *V. parahaemolyticus* abundance and percent pathogenicity are measured. For the epidemiological adjustment to the dose-response relationship, surveillance data on illness caused by *V. parahaemolyticus* from raw oyster consumption are collected.

These questions may be directly answered by primary research studies. Thus, eligibility criteria for studies are established a priori that clearly specify relevant study designs and can be identified using focused search strategies (EFSA 2010).

Harvest: Harvest questions examine environmental factors such as water temperature, turbidity, and dissolved oxygen and their influence on growth of *V. parahaemolyticus* and abundance in oysters at harvest. Harvest questions fall under the structure of questions about test accuracy.

1. Questions about test accuracy assess the accuracy and precision of methods used to detect and enumerate total and pathogenic *V. parahaemolyticus*, and expression of virulence factors.

a. Index test (I): methods used to detect and enumerate the target condition.
b. Target condition (T): abundance of total and pathogenic *V. parahaemolyticus* in water, sediment, and/ or oysters. Expression of virulence factors in water or oysters. Reference standard (culture MPN with PCR) used to decide if the target condition has been met.

Post-Harvest: Post-Harvest questions examine the effectiveness of PHPs at critical control points, after harvest and through the supply chain until retail. Post-Harvest questions fall under two basic structures, 1) questions about test accuracy; and 2) effects of an intervention.

1. Questions about test accuracy assess the accuracy and precision of methods used to detect and enumerate total and pathogenic *V. parahaemolyticus*, and expression of virulence factors.
   a. Index test (I): methods used to detect and enumerate the target condition.
   b. Target condition (T): abundance of total and pathogenic *V. parahaemolyticus* in oysters.
2. Questions on the effects of an intervention seek to understand the effect of PHP interventions on *V. parahaemolyticus* abundance through the supply chain.
   a. Population (P): raw oysters. In experimental studies, this may be in the form of shucked oysters, whole-shell oysters, or oyster homogenate.
   b. Intervention (I): types of PHPs applied alone or in combination, and levels of PHP interventions applied.
   c. Comparator (C): control of reference scenario, such as when the PHP has not been applied.
   d. Outcome (O): Total and pathogenic *V. parahaemolyticus* abundance (outcome) in raw oysters to indicate log reductions achieved.

Unintended effects include potential reductions to oyster texture, flavor, or other sensory qualities.

Consumption: consumption questions fall under the descriptive question structure.

1. Descriptive questions about consumption seek to understand consumption trends of specified consumers.

a. Population (P): How many servings do subpopulations of oyster consumers eat in a sitting? What characteristics make a person more likely to eat oysters, and how many oysters?
b. Outcome (O): Is exposure to *V. parahaemolyticus* a risk factor for illness in a certain population? Alternatively, is age, alcoholism, immunosuppression, liver disease, gender, or other health conditions a risk factor for illness following exposure to *V. parahaemolyticus* in raw oysters?

Table 2. Description and definition of the PICO statement. Based on EFSA (2010), OHAT (2015a) and the Wikoff et al. (2017) framework.

Populations of interest (P)	Humans. Consumers of raw oysters. Specific vulnerable populations include: immunocompromised persons; liver disease; alcoholism; the elderly. For animal feeding trials, animals would be the population of interest.
Exposure or intervention (E or I)	Exposure to *V. parahaemolyticus* in raw oysters; post-harvest practice interventions.
Comparator (C)	Lack of exposure to *V. parahaemolyticus* in raw oysters, or exposure to different dose levels of *V. parahaemolyticus* due to different harvest or post-harvest practices.
Outcome (O)	Risk of gastrointestinal illness, *V. parahaemolyticus* growth rates (model parameters).

Table 3. Inclusion and exclusion criteria.

	Inclusion Criteria	**Exclusion Criteria**
Publication Date	Published after 2004, or considered and not selected for the FDA 2005 QMRA.	Published before 2004 and/ or selected for the FDA QMRA
Category	Pertains to any of the three categories (dose-response, harvest or post-harvest population dynamics, oyster consumption behaviors).	Do not pertain to any categories
Data type / source	Data type: Primary research (epidemiological studies, randomized studies, cross-sectional), surveillance data, market surveys, systematic reviews, meta-analyses. Data source: journal articles in scientific or government databases, grey literature (government reports / white papers, dissertations, theses), expert opinion.	Not from an identified acceptable source
Biological relevance / plausibility	Studies on *V. parahaemolyticus*, or similar *Vibrio* spp. with findings biologically plausible / relevant for *V. parahaemolyticus*. Studies on oysters or other raw consumed shellfish whose findings are relevant to oysters.	Does not study a bacteria with biological plausibility for effect relevance to *V. parahaemolyticus*; does not study oysters or relevant species (other shellfish)
Region	Data collected in Chesapeake Bay, Washington, or region whose findings are generalizable to Chesapeake Bay or Washington state.	Data collected in region other than Chesapeake Bay or Washington whose findings are specific to that region.

Eligibility

Titles and abstracts were carefully reviewed for eligibility for full-text review and data extraction according to inclusion and exclusion criteria (Table 3) by two independent reviewers. Studies were screened out of further review if they met at least one category for exclusion using exclusion criteria (Table 4). Studies were further classified according to sub-categories of exclusion. Studies determined to meet the inclusion criteria by both reviewers underwent full-text review. Included studies were assigned to the following categories: dose-response, harvest, post-harvest, and/or oyster consumption behaviors (Table 5). Some studies were assigned to multiple categories.

Table 4. Categories for exclusion.

Reason Category	Definition
Accessibility	Unable to access full text without contacting the authors or for free
Bacteria genus or species mismatch	Not specific to *Vibrio parahaemolyticus*
Methods	Studies on the efficacy or development of a technical method(s)
Non-handling intervention	Excludes post-harvest practice and controls interventions (not relevant to the exposure route of interest)
Non-target exposure pathway	Exposure is not related to *Vp* in raw oysters
Other	Does not match any of the other categories
Oyster physiology	Oyster immune response and/or mortality
Review paper	
Vibrio mechanistic study	Examines mechanism of action of *Vp*[1], response to environmental conditions
Wrong animal species	Not oysters
Wrong outcome	Health outcome is not caused by the exposure pathway of interest (ingestion of raw oysters)
Wrong setting	Geographic location is not Chesapeake Bay or Washington State for harvest studies

[1]*Vibrio parahaemolyticus*

Table 5. Categories for inclusion.

Category	Examples of studies included
Dose-Response	Epidemiological surveillance studies, animal or human dose-response studies
Harvest	Studies on *Vp* prevalence in environment at harvest, response to environmental conditions (and change in conditions, such as due to climate change)
Post-Harvest	Studies on oyster post-harvest handling and processing practices and their effects on *Vp* growth
Consumption	Food consumption data, market surveys, consumer preference surveys

*Studies that fall meet multiple inclusion categories are recorded as such.

Full-text Review, Data Extraction

Data were extracted during full-text review from studies included by the two independent reviewers. One reviewer conducted full-text review, and a second reviewer confirmed decisions to exclude studies during full-text review, consistent with OHAT (2015a) guidance. Information on post-harvest models (and their associated parameters and inputs) were extracted and logged in Excel. To inform growth and survival of *V. parahaemolyticus* for the post-harvest model, data were extracted on PHP method(s) used, duration of treatment, level(s) of treatment, growth rate as a function of air temperature, PHP temperature, log reduction, *V. parahaemolyticus* levels during cold storage, and time in cold storage (Table 6).

Table 6. Information extracted for Post-Harvest Model.

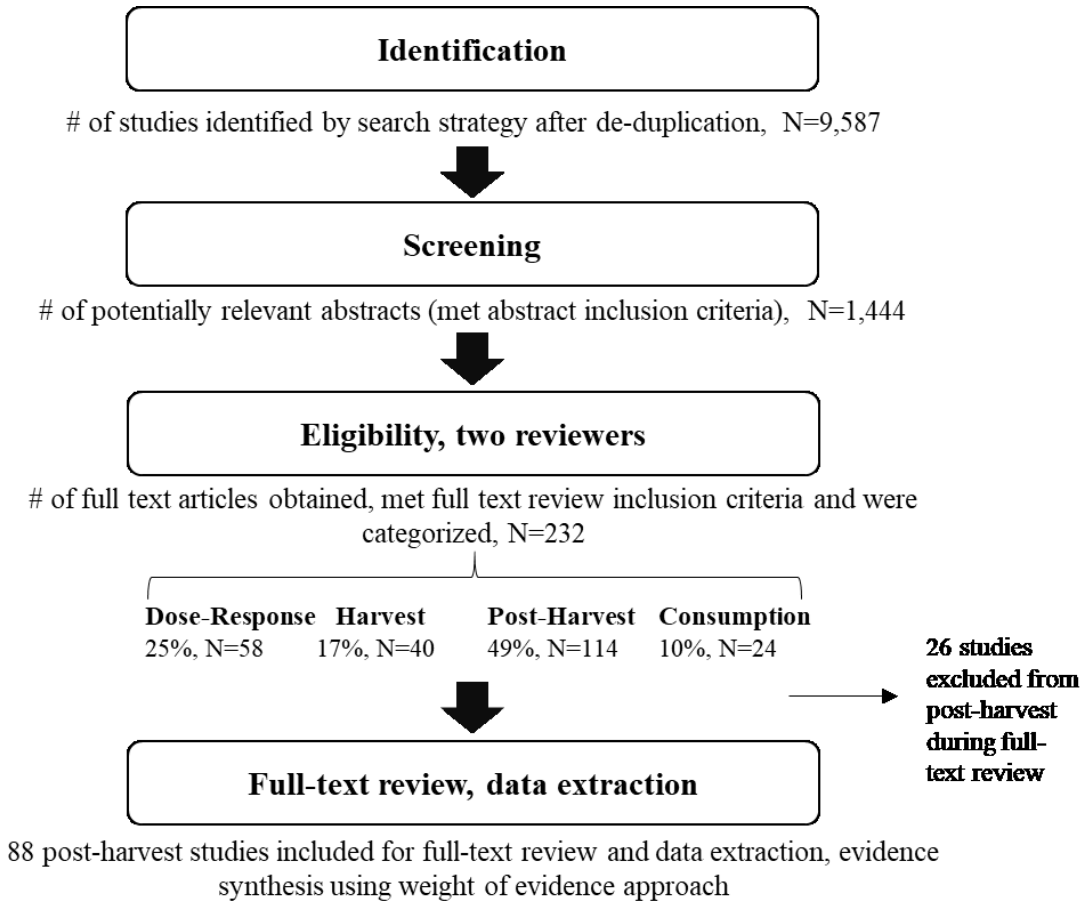
Data extracted	Post-Harvest Model
Post Harvest Process (PHP) information	PHP type(s) Season Location (or Experimental) Oyster species *Vp* Strain Serotype Virulence factor
Incubation information	Growth medium(s) Incubation temperature (°C)
Treatment conditions	PHP temperature (°C) PHP medium Time in treatment
PHP Efficacy	Pathogenic *Vp* at start Pathogenic *Vp* after PHP Growth rate Disinfection rate, %
Statistical measures	R^2 p value
Other	Qualitative findings

RESULTS

Flow of systematic review

The following schematic (Figure 3) depicts results from the identification, screening, and eligibility, and full-text review and data extraction phases:

Figure 3. Flow of Systematic Review.



16,557 studies were identified from all search strategies and databases and imported into Covidence from RefWorks. After removal of duplicates, 9,587 unique studies remained. 1,444 studies met at least one of the inclusion criteria in addition to date; these studies were considered potentially relevant. Two independent reviewers screened the titles and abstracts and, if

necessary, the full text, using the inclusion and exclusion criteria for full-text review. The most common reasons for excluding studies were because they were: methods papers, review papers, *Vibrio* mechanistic studies, or conducted in the wrong geographic setting. 232 studies were selected to be included for full-text review and data extraction during eligibility review. 58 studies were categorized as dose-response; 40 as harvest; 114 as post-harvest; and 24 as consumption (Figure 3). Note that studies may fall in multiple categories (Table 7).

For the dose-response studies, new human feeding trials were not anticipated to be identified. Animal model studies were identified. For consumption behavior studies, governmental reports and market surveys that include data on consumption patterns of oysters were identified. For harvest and post-harvest studies, research that assessed spatial (harvesting location) and temporal (season of harvest, time from harvest to refrigeration) variation in population dynamics of *V. parahaemolyticus* were identified. Identification of studies pertaining to harvest and post-harvest practices, with particular focus on the different intertidal methods used in Washington state, inform how those practices impact population dynamics and abundance of *V. parahaemolyticus* in oysters.

As most studies were categorized as post-harvest, full-text review and data extraction were first implemented on those studies and only these findings are reported in this thesis. During the full text review, 26 post-harvest studies were excluded as they met categories for exclusion; 88 were retained for data extraction, quality and relevance review, and evidence synthesis. See Table A7 in the Appendix for reasons for exclusion of post-harvest studies during full-text review. See Supplemental File 1 for all studies that were included for full-text review and data extraction for the dose-response, harvest, and consumption modules, and the post-harvest studies that were excluded during full-text review.

Table 7. Reasons for Exclusion.

Reasons for Exclusion	Number
Accessibility	57
Bacteria genus/ species mismatch	124
Duplicate	18
Methods	255
Non-handling intervention	80
Non-target exposure pathway	26
Other	80
Oyster physiology	25
Review	201
Vibrio mechanistic study	310
Wrong animal species	78
Wrong health outcome	10
Wrong setting	246
Total Unique Excluded	1212

Data Extraction

Qualitative findings are reported in Tables A8 – A20 in the Appendix. Quantitative study results on log reduction, survival or growth rate, or survival ratio, are reported here by post-harvest practice (PHP) method. Note that several studies assessing heat shock are in other tables where other PHPs were applied. Studies are listed by the first author and the year the study was published. For studies that investigated PHP efficacy on *V. parahaemolyticus* in oyster samples as well as in broth/ suspension/ milk/ a medium other than oyster tissue, only study results for *V. parahaemolyticus* in oyster samples are reported here. Detailed data extraction results can be found in the supplemental file. The following abbreviations and calculations were used:

- D-value = Decimal reduction time (minutes) or dose to kill 90% or 1 log of exposed bacteria
- D_P = Decimal reduction pressure (MPa), calculated as:

  $\log(N/N_0) = -(P/D_p) + a$

  where N_0 = initial number of cells, (CFU/ml); N = number of survivors (CFU/ml) after treatment at pressure P (MPa); D_P = increase in pressure (MPa) required for a 1 log reduction in number of cells; a = intercept (Chen 2006).
- LR = log reduction, calculated as lowest value reported (log reduction minimum mean – standard deviation) to highest value reported (log reduction maximum mean + standard deviation) for interventions.
- Survival rate or survival percentage = survival population / initial population x 100; reported as %
- Survival ratio: Log survival ratio = $\log_{10}(N_t/N_0)$

  where N_t = colony count after treatment; N_0 = colony count before treatment

Acid: Data extraction findings for acid post-harvest intervention studies are summarized in Table 8. Acids such as lactic, citric, acetic, and tartaric acid are commonly used in food preparation and processing. In using acid as a lethal environmental stressor to inhibit *V. parahaemolyticus* growth, Chiang 2009 found that cell growth phase affected *V. parahaemolyticus* survival when exposed to lethal acid. Strains and virulence genes have been shown to play a role in *V. parahaemolyticus* acid resistance: strains that had virulence genes and strains that were isolated from cold temperature seawater sources exhibited significantly greater resistance to / tolerance of acid (Hasegawa 2013). Kalburge (2014) also found differences in survival rates to acid stress across different strains.

V. parahaemolyticus cells previously exposed to sub-lethal acid challenges may develop an acid adaptation response, in which mild acid preadaptation significantly increases survival to lethal acid stress (Kalburge 2014). Yeung (2004) demonstrated that survival of acid adapted or non-adapted cells to acid exposure varied according to cell phase. Acid adaptation responses have been shown to vary among strains of *V. parahaemolyticus* and affect *V. parahaemolyticus* survival to subsequent application of other mitigation strategies such as disinfectants or high salinity (Chiang 2014). Chiang (2008a) found that *V. parahaemolyticus* cells had the highest resistance to acetic acid, followed by lactic acid, citric acid, and tartaric acid. Ethanol shock increased subsequent *V. parahaemolyticus* susceptibility to acid challenge and made cells most susceptible to acetic followed by lactic acid, though reductions were still less than 3.52 logs (Chiang 2008a). Cold shock reduced *V. parahaemolyticus* resistance to lactic acid; though there was not a significant difference in survival, cold-shocked cells were inactivated more rapidly with exposure to lactic acid than cells which had not been cold-shocked (Lin 2004). Cold shock at 15°C proved more effective at reducing acid tolerance of *V. parahaemolyticus* that cold shock at 20°C.

Note that Hasegawa 2013 and Kalburge 2014 were included studies on acid stress that did not report quantitative findings; see qualitative findings for those and other acid studies in Table A8 in the Appendix.

Table 8. Data Extraction: Acid studies.

Study	PHP Method	Log reduction (log CFU/ml)	Survival rate (%)
Chiang 2008a	Organic acids	0.52 to 0.87	
Chiang 2008a	Ethanol shock, organic acids	0.89 to 2.42	
Chiang 2009	Acid, acid adaptation	4.5 to 5	14.79 to 27.06
Chiang 2014	Acid adaptation, Heat stress		4.2
Chiang 2014	Acid adaptation, Ethanol		32.7
Chiang 2014	Acid adaptation, High salinity		3.5
Chiang 2014	Acid adaptation, Hydrogen peroxide		1.2
Lin 2004	Lactic acid		.16
Lin 2004	Acid, cold shock		.02
Lin 2013	Acid adaptation, Cl disinfectant	1.20 to 4.95	
Whitaker 2010	Acid	2 to > 6	
Wong 2004	Acid		50
Yeung 2004	Acid stress	.1 to 4.3	

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Cold storage: Data extraction findings for cold storage studies are summarized in Tables 9 and 10. Survival and reduction rates varied according to storage temperature. Storage at -18°C achieved greater reductions in survival of *V. parahaemolyticus* (3.2 survival ratio) than storage at 5°C for 8 days (38 survival ratio) (Chang 2004). Though increasing storage duration has been shown to decrease survival, more time spent in cold storage may negatively affect the quality of oyster meat for consumption. Additionally, slow cooling typical of oyster industry practices may increase resistance to mitigation strategies (DePaola 2009).

Pathogenic cells positive for *tdh* and/or *trh* subjected to starvation during cold storage had reduced survivability and recovery compared to non-pathogenic cells; this may help explain the difficulty in recovering more pathogenic strains from shellfish and highlights an important limitation of widely used detection and enumeration methods (Drake 2008). Additionally, pathogenic strains with the *trh* gene exhibited poor survival during cold storage, though log reduction was less than 1 (Liao 2017). The freezing and frozen storage process used by Liu 2009 found reductions in *V. parahaemolyticus* exceeded 3 logs after 3 months of storage and exceeded 3.5 log reductions after 5 months of storage. A validation process confirmed the efficacy of the National Shellfish Sanitation Program (NSSP) guidance (Liu 2009). Growth rate of *V. parahaemolyticus* during cold storage has been shown to vary across different species of oysters, in addition to different strains. Mudoh (2010) found no growth of pathogenic *V. parahaemolyticus* in Eastern oysters at 5°C but found slow inactivation in Asian oysters at the same temperature (Table 9). As such, incorporation of oyster species as well as *V. parahaemolyticus* strain and serotype can be used to inform and improve predictability of the post-harvest risk model.

Overall, differential growth and survival responses across strains to cold storage treatments (Burnham 2009) pose important implications for the post-harvest model, in that incorporating strain differences would enhance predictions of *V. parahaemolyticus* survival

during cold storage. Qualitative findings of Songsaeng 2010 and other cold storage studies are in Tables A9 and A10 in the Appendix.

Table 9. Data Extraction: Cold Storage studies. Part 1.

Study	PHP Method	Log reduction (log CFU/ml)	Survival rate (%) or D-value	Growth rate
Burnham 2009	Cold storage	1.67 to 3.34		
Chang 2004	Cold storage		3.2 to 38 %	
Chang 2004	Heat shock, Cold storage		0.1 to 17 %	
Chiang 2006	Cold storage		74.2 %	
Chiang 2006	Ethanol shock, Cold storage		70.2 to 72.2%	
Chiang 2006	Frozen storage		1.59%	
Chiang 2006	Ethanol shock, frozen storage		0.41 to 0.50%	
DePaola 2009[1]	Freezing, frozen storage	Final density: < .04 – 3 to > 30 MPN /g		
DePaola 2010[2]	Cold storage	Final density: $\leq$ 1 to > 10^5 MPN/g		
Drake 2008[3]	Cold storage; Starvation, cold stress		D-value: 4.1 to 7.5 (days)	
Fernandez-Piquer 2010	Cold storage			.036 to .205 log MPN/hr
Fernandez-Piquer 2011	Cold storage	2.4		
Huang 2018	Cold storage	0.8 to 1.3 log CFU/g (at 12 to 4 C)		
Jones 2017	1 hr ambient storage then refrigerated	0 log MPN/g		
Jones 2017	5 hr ambient storage then refrigerated	Increase: 1.6 log MPN/g		
Jones 2017	7 hr shade storage then refrigerated	Increase: 0.9 log MPN/g		
Liao 2017	Cold storage	0.213 to 2.109 log CFU/g		
Lin 2004	Cold storage		1.98 to 14%	
Lin 2004	Cold shock, cold storage		32.1%	

Table 10. Data Extraction: Cold Storage studies. Part 2.

Study	PHP Method	Log reduction (log CFU/ml)	Survival rate (%) or D-value	Growth rate
Liu 2009	Freezing, frozen storage	2.53 to 4.55 log MPN/g		
Mudoh 2010[4]	Cold storage			-.0007 to -.0019 (total); .048 to .17 (*tdh*+) log CFU/hr
Mudoh 2014	Cold storage	O to increased growth (4 log CFU/g)		
Parveen 2013	Cold storage, HHP			-.0036 to .022 log CFU/hr
Prapaiwong 2009	Cold storage	2.95 log CFU/g		
Prapaiwong 2009	Quick frozen, cold storage	2.59 log CFU/g		
Shen 2009	Cold storage	LR: 2 to 2.38 log MPN/g for 5 to 10C; increase of 1.62 log MPN/g at 15C		
Vasudevan 2006	Cold storage	< 1 to 3		
Wang 2018a	Cold storage	2.11 log CFU/g increase at 15 C		
Wu 2007	Cold storage	1.4 to 2.21		
Wu 2007	Ice water storage	1.3 to 2.09		
Ye 2013	Cold storage, HHP	0 to < 6 log MPN/g		
Zarei 2014	Cold storage		12.6 % (total), 17.4 % (*tdh*+)	
Zarei 2014	Cold storage, Chlorine stress		1.1 % (total), 1.2 % (*tdh*+)	

[1]Final values reported as this was a market survey and initial values were not reported.

[2]Final values at retail reported, market survey so initial values not reported.

[3]D-value for Drake 2008 was calculated as $D = 2.94 / \beta$

[4]Total *Vp* only tested at 5°C. 10 and 15°C were other temperatures tested considered to be cold storage with results listed for pathogenic *V. parahaemolyticus*.

Depuration: Data extraction findings for depuration studies are summarized in Table 11. Effectiveness of depuration as a post-harvest mitigation strategy was shown to vary across seasons (Yu 2010), and to vary by strain, size and type of oysters. In Pacific oysters during the winter, 3-log reductions in *V. parahaemolyticus* were met after 96 hours of depuration at 5°C; in Pacific oysters during the summer, 144 hours were required to reach the same log reduction (Su 2010). Oyster physiology also plays a role in efficacy of depuration and other post-harvest practices. Oyster biological activity was found to be minimized at temperatures less than 5°C in Pacific oysters (Phuvasate 2012). In addition to temperature, salinity affects oyster ability to clear *V. parahaemolyticus*. Salinity greater than or equal to 20 parts per thousand (ppt) proved favorable for oyster physiology and filter feeding activity. Greater log reductions were observed in Pacific oysters that underwent depuration in seawater between 20 and 30 ppt, than in salinity of 10 ppt (Phuvasate 2013).

As reductions were overall not consistently found to meet the 3.52 logs required for a validated PHP (NSSP 2017), higher initial values of *V. parahaemolyticus* present during warmer months restrict the use of depuration as an effective strategy (Table 11). Though decreasing water temperature has been shown to increase log reduction in *V. parahaemolyticus* achieved through depuration, the effect was found to be limited below 15°C; depuration at 15°C was more efficient than at 22°C, but depuration at 10°C did not result in significantly greater reductions than at 22°C in Gulf oysters (Chae 2009). Larsen (2015) also found that depuration conducted at lower temperatures (20°C) tended to be less effective than depuration conducted at higher temperatures in Eastern oysters, such as at 22.5 and 25°C.

Log reduction from depuration also varied significantly between type and size of Pacific oysters (Phuvasate 2013); such findings should be considered in informing regulatory practices to better account for oyster characteristics that increase susceptibility to persistent *V. parahaemolyticus* populations. Regarding virulence factors that promote pathogenicity and resistance to PHPs, Aagesen (2013) found that *V. parahaemolyticus* uses type I and type IV pili to

persist in the Pacific oyster during depuration, and both polar and lateral flagellar systems are important. The digestive glands and gills were found to be good sample sites for direct monitoring of *V. parahaemolyticus* in oysters, as *V. parahaemolyticus* abundance was highest in the digestive glands, followed by the gills (Wang 2010b).

Chae 2009 found that increasing depuration time to 4 days increased *V. parahaemolyticus* reductions to 2.6 log MPN/g, but it still did not meet the NSSP recommended log reduction of 3.52 (Table 11). Larsen 2015 showed that high salinities reduced *V. parahaemolyticus* to less than 30 MPN/g, but longer depuration times (greater than 10 days) may be necessary to consistently reach greater than three log reductions. Based on reduction rates from depuration trial results conducted over 6 days, Ming 2018 predicted that depuration would need to be extended beyond 8 days to meet greater than 3.52 log reductions. In applying various sterilization techniques to depuration, Ramos 2012 demonstrated no significant difference in log reduction achieved between applying UV light versus UV light and Chlorine. UV was sufficient to achieve a 3-log reduction in Pacific oysters (Ramos 2012). Overall, depuration was not shown to have variability in effectiveness and did not meet the threshold for post-harvest. Qualitative findings of depuration studies are in Table A11 in the Appendix.

Table 11. Data Extraction: Depuration studies.

Study	PHP Method	Log reduction (log CFU/ml)	Survival rate (%)
Aagesen 2013	Depuration	3.35 to 4.79 log CFU/g	
Aagesen 2014	Depuration, heat shock	0 or increase	
Chae 2009	Depuration	0.25 to 2.09 log MPN/g	
Larsen 2015	Depuration, high salinity	3.95 to 5.01 log MPN/g	
Ming 2018	Depuration; depuration with algae feeding	2.47 log MPN/g; 2.19	
Phuvasate 2012	Depuration with UV	1.31 to 3.4 log MPN/g	
Phuvasate 2013	Depuration	2.07 to 3.28 log MPN/g	
Phuvasate 2013	Depuration	Diploid: XS: 3.26 ± 0.23 M: 2.91 ± 0.34 Triploid: XS: 2.90 ± 0.35 M: 3.07 ± 0.36 Log MPN/g	
Ramos 2012	Depuration, UV light, Cl	2 to 3.1 log MPN/g	
Sobrinho 2014	Depuration with ozone, UV	-1.1 to .3 MPN/g	
Su 2010	Depuration	2.57 to 3.09 log MPN/g	
Wang 2010b	Depuration		1.4 to 28.1%
Yu 2010	Depuration and recirculation or flow-through, creek relay	0.96 to 2.84 log MPN/g	

Disinfectants: Data extraction findings for disinfectant studies are summarized in Table 12. Studies investigating disinfectants such as ethanol, hydrogen peroxide, and sodium chloride showed overall that such treatment can be an effective strategy for reducing and preventing cross-contamination (Table 12). Use of electrolyzed oxidizing (EO) water for 30 to 60 seconds completely inactivated *V. parahaemolyticus* on stainless steel, glazed ceramic tile, and plastic cutting board (Chiu 2006). However, EO water was less effective on bamboo or wood cutting boards (Chiu 2006). Chiang 2009 found that susceptibility of *V. parahaemolyticus* to lethal heat stress with ethanol was dependent on cell growth phase, with stationary phase cells having a higher survival rate than mid-exponential phase cells. Wang 2010a applied chlorine dioxide treatment to examine *V. parahaemolyticus* retention in oyster homogenates as well as in different oyster tissues. In addition to use on surfaces, disinfectants can also be applied to seafood. Wang 2010a found that *V. parahaemolyticus* can be completely disinfected from oyster tissues after 6 hours of treatment. Shi 2017 found that saline solution achieved greater log reductions on stainless steel than polypropylene surfaces. Average recovery rates were higher for polypropylene than stainless steel following saline solution application (Shi 2017).

Chang 2004, Chiang 2008b and Lai 2013 examined disinfectant use but not did not report quantitative or easily extractable findings for disinfectants; see Tables A12 and A13 in the Appendix for qualitative results for those and other disinfectant studies.

Table 12. Data Extraction: Disinfectant studies.

Study	PHP Method	Log reduction (log CFU/ml)	Survival rate (%)
Chiang 2006	Ethanol shock	0 to 0.38	
Chiang 2006	Ethanol shock, heat shock		0.31 %
Chiang 2008a	Ethanol shock	.75 to .93	10.6 to 15.8 %
Chiang 2008a	Ethanol shock, organic acids	.87 to 2.20	
Chiang 2008a	Ethanol shock, Hydrogen peroxide		2.5 %
Chiang 2008a	Ethanol shock, NaCl		0.5 to 5.9%
Chiang 2009	Ethanol shock	2.5 to 3.5	
Chiang 2014	Ethanol, acid adaptation		10.4%
Chiu 2006	Electrolyzed oxidizing water on surfaces	4.02 to > 5	
Lin 2004	Hydrogen peroxide		< .01%
Lin 2004	Hydrogen peroxide, cold shock		0.55 %
Lin 2013	Chlorine disinfectant	4.28 to 6.84	
Lin 2013	Chlorine disinfectant, heat shock	1.21 to 5.52	
Lin 2013	Chlorine disinfectant, cold shock	1.33 to 5.41	
Park 2018	Sodium hypochlorite	0 to 2	
Park 2018	Sodium hypochlorite, Gamma irradiation	5 to > 5.6	
Shi 2017	Saline solution on surfaces	4.74 to 7.15 log CFU/cm^2	
Takahashi 2016	Sodium hypochlorite	0.03 to 0.79 – inactivation rate	
Wang 2010a	Chlorine dioxide	2.3 to 3.1 log CFU/g	
Zarei 2014	Sodium chloride		14.5 to 19.3%
Zarei 2014	Chlorine stress, sodium chloride		4 to 5.6%

High Hydrostatic Pressure (HHP): Data extraction findings for HHP studies are summarized in Table 13. In comparison to other foodborne pathogens, *V. parahaemolyticus* has been shown to be sensitive to high pressure treatments (Chen 2007). In a survey of market oysters, over 70% of samples that had received HHP treatment were reduced to less than .04 log MPN/g (DePaola 2009). Resistance to pressure treatment varied among strains (Phuvasate 2015). Strains with the O3:K6 serotype were the most pressure resistant of serotypes tested in inoculated Eastern *Crassostrea virginica)* oysters (Koo 2006). Though treatment of 300 MPa for 5 minutes completely inactivated all clinical strains to less than the limit of detection, ranging from 6.2 to 7.7 log reductions, strains responded differently to lower pressure treatments. After 200-250 MPa treatment, reductions observed in 5 clinical strains differed by up to 3.1 log CFU/ml, highlighting the wide variability in efficacy by strain of *V. parahaemolyticus* (Phuvasate 2015). Studies comparing results in oyster meat versus whole-shell oysters show that whole shell oyster results are comparable to results for oyster meat (Ye 2013), indicating that lab studies conducted on oyster homogenates are generalizable to HHP as an effective PHP for whole-shell oysters.

Data suggest that the log number of survivors has a linear relationship with pressure applied (Chen 2006), with Weibull frequency distributions predicting inactivation from HHP more accurately than other distributions in oyster samples and pure cultures alike (Hu 2005) (Table 13). Ma 2011 found that application of 293 MPa for 120 seconds at about 8°C could achieve at least 3.52 log reductions in *V. parahaemolyticus*. This treatment was considered the minimum to achieve the required log reductions post-harvest. Phuvasate (2015) showed that treatment of 250 MPa for 5 minutes at 1.5°C inactivated greater than 6.5 log *V. parahaemolyticus* in suspension, and greater than 6.4 log of all three strains tested in oyster homogenates. *V. parahaemolyticus* strain 10290 was most resistant, while strain 10292 was most sensitive, to pressure treatments (Phuvasate 2015). In validation studies conducted by Ye (2013) in Eastern oysters, treatments of 250 MPa followed by 10 day ice storage, at 300 MPa followed by 5 day ice storage, and at both pressure levels followed by 7 day frozen storage completely eliminated *V.*

parahaemolyticus in whole-shell oysters. Overall, studies support the use of HHP as an effective PHP to reduce risk of illness from raw oyster consumption. Qualitative findings are listed in Table A14.

Table 13. Data Extraction: HHP studies.

Study	PHP Method	Log reduction (log CFU/ml)	D_P (MPa)	D-value (min)
Chen 2006	HHP	2.8 to 6.1	17.1 to 21.8	
Chen 2007	HHP	3.4 to 7.6		
DePaola 2009	HHP	.04 - > 5 log MPN/g		
Hu 2005	HHP			2.09 to 4.19 -oyster homogenate-Arrhenius results
Koo 2006	HHP	4.3 to 5.7 log CFU/g		1.8 to 3.5
Kural 2008	HHP	5 to 8.7		
Kural 2008	HHP, varying temp	4 to 7.7		
Ma 2011	HHP	2.7 to > 3.53 log MPN/g		
Phuvasate 2015	HHP	1.9 to > 7.1		
Phuvasate 2015	HHP, low temp	1.4 to > 7.2		
Prapaiwong 2009	HHP, storage	Increased during storage		
Vu 2018	HHP	1.1 to > 6		
Ye 2011	HHP, incubation temp.	4.2 to 6.7 log CFU/g		
Ye 2012	HHP	1.6 to 7.8 log MPN/g		
Ye 2012	HHP, mild heat	3.6 to < 7.6 log MPN/g		
Ye 2013	HHP	5 to 6.9 log MPN/g		
Ye 2013	Storage (varying temp) then HHP	3.9 to 7.4 log MPN/g		
Ye 2013	HHP then cold storage	0 to > 2.7 log MPN/g		

Icing: Data extraction findings for icing post-harvest intervention studies are summarized in Table 14. Study findings are contradictory regarding the effectiveness of icing in controlling *V. parahaemolyticus* post-harvest (Table 14). Data do not support the efficacy of rapidly cooling oysters using ice on board immediately after harvest during warmer months for Eastern oysters (Thomas 2016). However, Lydon (2015) findings supported the use of ice slurries to rapidly cool oysters. Jones (2017) found consistently but not statistically significantly lower *V. parahaemolyticus* abundance in oysters immediately iced after harvest, compared to oysters that were insulated from direct contact with ice during post-harvest transport, the current recommended method for collection and transport. Temperature monitors found that oysters remain at temperatures conducive for *V. parahaemolyticus* growth for 3 to 5 hours after harvest if not placed directly in contact with ice (Jones 2017). These findings may have important implications to future revisions of *V. parahaemolyticus* control plans.

Log reductions were not consistently achieved across all studies. Further, icing negatively affected Eastern oyster survival during subsequent cold storage (Melody 2008a). Qualitative findings of icing studies are listed in Table A15.

Table 14. Data Extraction: Icing studies.

Study	PHP Method	Reduction	Increase
Jones 2017	Immediate icing	0.4 log MPN/g	
Jones 2017	Icing after 1 hr ambient storage	0.3 log MPN/g	
Jones 2017	Icing after 5 hr ambient storage		0.8 log MPN/g
Melody 2008a	On-board or dockside icing, cold storage		0 to 2 log CFU/g
Lydon 2015[1]	Ice slurry		2.74 log MPN/g (total) .09 MPN/g (*tdh*+); 0.16 MPN/g (*trh*+)
Thomas 2016	On-board icing		0 to <1 MPN/g (pathogenic)

[1]Median values reported.

Irradiation: Data extraction findings for icing studies are summarized in Table 15. Irradiation has the potential to reduce *V. parahaemolyticus* populations by more than 5 logs under certain conditions (Table 15). Of concern, *V. parahaemolyticus* strains respond differently to irradiation based on expression of certain virulence genes. Gamma-irradiation can reduce or enhance virulence (Abdallah 2009). While an increase in the *toxS* gene was observed after treatment, the *toxR* gene remained stable (Abdallah 2009). A nonlinear relationship of microbial survival curves to irradiation exposure was found (Hu 2005). Simultaneous irradiation with UVA and UVC indicated a synergistic bactericidal effect (Nakahashi 2014). Mahmoud 2009 found evidence for a differential dose-response relationship between irradiation exposure and log reductions based on type of Eastern oyster: a higher dose was needed to reduce *V. parahaemolyticus* to less than the limit of detection in whole shell than in half shell oysters, as oyster shells decrease the maximum deliverable dose rate by 35% (Mahmoud 2009).

Data suggest 60 ppm of NaClO in combination with 2.0 kGy effectively reduces *V. parahaemolyticus* by 7 logs without any deteriorative changes of sensory qualities, supporting its use as an effective PHP in Pacific oysters, *Crassostrea gigas* (Park 2018). Thulipa (2011) also found no significant differences in overall acceptability between controls and irradiated oysters during a sensory evaluation. Overall, irradiation was found to be an effective PHP with negligible impact to consumer experience.

Abdallah 2009, Andrews 2011, and Jin 2009 did not report quantitative results; qualitative findings of these and other irradiation studies are reported in Table A16 in the Appendix.

Table 15. Data extraction: Irradiation studies.

Study	PHP Method	Log reduction (log CFU/ml)	D-value (min)	Survival rate (%) or ratio
Hou 2016	UVA Irradiation			< -1 log survival ratio
Hu 2005	HHP, Gamma Irradiation		0.22	50% at .57 kGy
Mahmoud 2009	X-ray irradiation	1 to > 7 log MPN/g		
Nakahashi 2014	UV Irradiation			-3.9 to -.96 log survival ratio
Park 2018	Gamma irradiation	1 to 4.2 log CFU/g		
Park 2018	NaClO, gamma irradiation	5 to > 5.6 log CFU/g		
Thupila 2011	Irradiation	0.55 to 4.38 log CFU/g		
Yagi 2007	UV-LED Irradiation			0% (100% inactivation rate)

Other types of PHP interventions: The following types of PHPs were not easily classifiable into the other categories of interventions, so are reported as 'Other.'

D-Tryptophan: Data extraction findings for D-Tryptophan are summarized in Table 16. D-Tryptophan is a bacterial metabolite and D-amino acid that may exhibit antibacterial properties (Chen 2018). Though D-Tryptophan did not achieve 3.52 log reductions in *V. parahaemolyticus*, it has the potential to control *V. parahaemolyticus* populations in live oysters in artificial seawater, as higher salinity levels had greater inhibitory effect of *V. parahaemolyticus* growth. D-Tryptophan may also have the potential to extend the shelf life of shucked oysters in artificial seawater, when held at refrigeration temperatures (Chen 2018). Though other antibacterial agents were not considered for this review based on limited time and resources, future reviews may examine the role that other antibacterial agents may have as alternative mitigation strategies.

Low salinity stress: Findings of lethal low salinity challenge varied across strains and virulence factors (Huang 2012). Survival to lethal low salinity was strain dependent, with surviving populations ranging from 0.7 to 3.6 log CFU/ ml (Huang 2012). A majority (77 to 84%) of cells were estimated to be in the VBNC state at 4 to 10 minutes, respectively (Huang 2012).

Qualitative findings of Chen 2018, Fujikawa 2009 are in Table A17 in the Appendix.

Table 16. Data Extraction: Other PHP Interventions.

Study	PHP Method	Log reduction	Log increase
Chen 2018	D-Tryptophan	2.2 log CFU/g	
Chen 2018	D-Tryptophan, salt, cold storage	2.7 log CFU/g (broth)	
Huang 2012[1]	Low salinity stress	2.3 to 6 (*tdh*-); 3.7 to 6.9 *tdh*+); 2 to 6 (*trh*+) log CFU/ml	
Wang 2018b	Food matrix		5.91 to 6.51 (*tdh*+); 5.8 to 6.8 (*trh*+) log CFU/g

[1]Initial – (Mean ± SD survival)

Relaying: Data extraction findings for relaying studies are summarized in Table 17. High salinity relay conducted in the Chesapeake Bay during the summer reduced abundance of *V. parahaemolyticus* in Eastern oysters, *Crassostrea virginica*, but did not significantly affect virulence genes (Elmahdi 2018). High salinity relay conducted in refrigerated seawater (RSW) tanks at 15.5°C and 8 ppt salinity reduced *V. parahaemolyticus* abundance by approximately 1.9 logs over 7 days; at 17 and 34 ppt, *V. parahaemolyticus* abundance increased (Jahnke 2011). When oysters were first held at 22.2°C for seven hours and then transferred to RSW tanks at 8 ppt salinity, numbers decreased by approximately 3.2 logs at 8 ppt for 6 days and at 17 ppt for 12 days. Salinity levels of 34 ppt also tended to increase with this method (Jahnke 2011). Overall, relay at lower salinities proved more effective at reducing *V. parahaemolyticus* growth than at high salinity levels in RSW tanks (Jahnke 2011).

However, effect of salinity on relay efficacy was inconsistent across studies and varied according to experimental setting (Table 17). Melody 2008b found that moving Eastern oysters from areas of medium salinity, 15 ppt, to areas of higher salinity, 30 ppt, significantly reduced *V. parahaemolyticus* abundance. After one week of relay at the site, counts did not differ significantly from samples that were iced immediately after harvesting, though levels were still higher than the limit of detection (< 1 CFU / 0.1 g) (Melody 2008b). Parveen (2017) also found that reductions in *V. parahaemolyticus* in Eastern oysters were more consistent at high salinity sites in the Maryland portion of Chesapeake Bay, after 14 and 21 days. Statistically significant differences of more than 1 and 2 logs were observed after 21 and 28 days of storage at 32 to 34 ppt, respectively. All treatments achieved greater than 2.6 log MPN/g reductions but were less than 3.52 (Parveen 2017). Relaying of Eastern oysters to relatively higher salinity waters also demonstrated potential for reducing abundance at initial harvest after 5 days based on preliminary results (Walton 2013).

Some of the relaying studies were observational by design and conducted in the shellfish harvesting environment. In the Taylor (2018) study, levels of *V. parahaemolyticus* at the harvest

study site were not high enough to demonstrate multi-log reduction during relaying, so naturally contaminated oysters were temperature abused to allow initial levels to increase. Subsequently, 4 log reductions were met after 14 days of relaying Eastern oysters (Taylor 2018). Yu 2010 found that high salinity relaying to a creek achieved greater than 1 log reductions, but reductions were not significant.

Elmahdi 2018 and Walton 2013 reported qualitative results, listed in Appendix Table A18 with other qualitative findings of relay studies.

Table 17. Data Extraction: Relay studies.

Study	**PHP Method**	**Log reduction**	**Log increase** (in counts)
Jahncke 2011	High salinity relay	1.88 in counts	2.53 to 2.57
Jahncke 2011	Storage, High salinity relay	3.17 in counts	2.95 to 3.49
Parveen 2017	High salinity relay	1 to > 2 log MPN/g	
Taylor 2018	High salinity relay	4.5 log MPN/g	
Yu 2010	High salinity relay	1.57 to 2.84 log MPN/g	

Re-submersion: Data extraction findings for re-submersion post-harvest intervention studies are summarized in Table 18. Overall, findings support a minimum 7-day re-submersion regimen to allow *V. parahaemolyticus* levels to return to levels at initial harvest (Kinsey 2015, Grodeska 2017). This finding was based on time necessary for *V. parahaemolyticus* to decrease to levels not significantly different from controls (continually submersed oysters) after a period of air drying for 27 hours or 3 hours of freshwater dipping followed by 24-hour air drying of Eastern oysters (Grodeska 2017) before being exposed to re-submersion. Levels of *tdh* were significantly greater than background levels after 7 days (Kinsey 2015). However, *tdh* levels did not differ significantly after 14 days of re-submersion (Kinsey 2015). Re-submersion proved to be more effective and rapid with high initial numbers of *V. parahaemolyticus*. Qualitative findings of studies on re-submersion are in Table A19.

Table 18. Data Extraction: Re-submersion studies.

Study	PHP Method	Log reduction (log MPN/g)
Grodeska 2017[1]	Desiccation, re-submersion	.03 log reduction or 0.3 to 1.4 log increase
Jones 2016	Intertidal exposure, re-submersion	2.73 to 3.21 (total), < 1 (*tdh*+), 2 (*trh*+)
Kinsey 2015	Dry storage, re-submersion	0.2 to 0.3 log reduction or 0.2 to 0.9 log increase

[1]Data from trial 4

Temperature shock and stress: Data extraction findings for temperature shock and stress studies are summarized in Table 19. *V. parahaemolyticus* strains responded differently to heat shock, with strain 690 demonstrating a significant increase in thermal tolerance as duration of heat shock increased (Chang 2004). The source of pathogenic strains also affected heat shock response; thermal tolerance of clinical strains positive for *tdh* and / or *trh* was significantly higher than the heat tolerance of strains isolated from the coastal environment and seafood that were *tdh*+ and /or *trh*+ (Hasegawa 2013). However, there was no significant difference observed in tolerance against free-thaw stress between *tdh*-and *trh*- strains, and *trh*+ and/ or *trh*+ strains (Hasegawa 2013).

Environmental conditions affected *V. parahaemolyticus* response to temperature shocks and stresses. While cells pre-adapted in 3% NaCl exhibited greater survival than cells preadapted in 6% NaCl when subjected to cold stress, cells subjected to heat stress demonstrated the opposite response: cells pre-adapted in 6% NaCl had higher survival than cells pre-adapted in 3% (Kalburge 2014). Whitaker (2010) also found that cells grown in 1% NaCl were less resistant to heat and cold stress than cells grown at 3% NaCl, further supporting the use of limiting conditions that support growth. In combination, low salinity and low temperature also significantly reduce *V. parahaemolyticus* survival (Lai 2013).

Heat and cold shock have important implications for the effectiveness of PHPs. Notably, heat shock significantly affects the efficacy of depuration in Pacific oysters in some but not all months (Aagesen 2014).

Growth rate and lag time were found to vary based on temperature and strain (Kim 2012). Though *V. parahaemolyticus* strain 33844 was the fastest growing among other strains tested, its lag time (10.32 hr) was greater than the lag time predicted for pathogenic (8.16 hr) and nonpathogenic (6.25 hr) *V. parahaemolyticus* isolated from raw oysters (Kim 2012). Consideration of a lag phase modeled with the Gompertz (Kim 2012) or other biologically plausible equation is a distinction from the existing QMRA. Specific growth rates of pathogenic

V. parahaemolyticus were 0.42 log CFU/hr, and nonpathogenic *V. parahaemolyticus* were 0.42 log CFU/ml.

Of note, several studies assessing heat shock are in other PHP data extraction tables because other PHPs were also applied. Chiang 2008b, Hasegawa 2013, Kalburge 2014 and Nishina 2004 reported qualitative reports, those are listed in the Appendix Table A20 with other qualitative findings of temperature shock and stress studies.

Table 19. Data Extraction: Temperature shock and stress studies.

Study	PHP Method	Log reduction (log CFU/ml)	Survival rate (%)	Growth rate (log CFU/hr)
Aagesen 2014	Heat shock	Increase < 1 (growth)		
Chang 2004	Heat shock	< .5		
Chang 2004	Heat shock, heat stress		0.3%	
Chiang 2006	Heat shock		0.03%	
Chiang 2009	Heat shock	~6	0.32 to 2.24 %	
Chiang 2014	Heat stress		0.2%	
Chiang 2014	Cold stress		42.6 to 53.1%	
DePaola 2009	Mild heat	0 to 3	>90% of the mild heat and HHP samples had > 0.04 *Vp* per g, 750-fold lower than specified for nondetectable levels (<30/g)	
Kim 2012	Moderate temp[1]			0 to 0.3
Kim 2012	High temp[2]			0.54 to 1.53
Lai 2013	Cold stress, low salinity, starvation	3 to 4		
Whitaker 2010	Acid stress, cold stress		1 to 65 %	
Zarei 2014	Heat shock		1.1 to 2.4%	

[1]13 to 18°C

[2]24 to 36°C

DISCUSSION

Overall, there was substantial coverage in the literature on acid, cold storage, depuration, HHP, irradiation, disinfectants, relay, and temperature shock or stress interventions. Literature on icing and re-submersion were limited. Results from data extraction have important implications for future efforts to develop regionalized QMRAs, detailed in chapters 3 and 4. While this chapter has explored and reported the current state of the evidence on the effectiveness of selected PHPs in reducing *V. parahaemolyticus* abundance, it is also necessary to evaluate the quality of the study designs and methods used to reach those results. As such, Chapter 3 will evaluate the level of confidence in the results reported here, as measured by study quality criteria. The relevance of these results to the purpose of the project, in providing inputs to support a future QMRA on risk of illness from *V. parahaemolyticus* in raw oysters harvested in the Chesapeake Bay and Washington state, will also be evaluated in Chapter 3.

A key strength of this approach was that the PICO statement defined the type of data extracted from studies and specified in the data extraction protocol according to PICO. Such methodology promotes transparency of the process and reproducibility of the findings.

Key limitations to the data extraction approach involved the type and quantity of the data reported, which made interpreting and extracting data from the wide variety of types of information provided in the studies more difficult. The extent to which a study reported the pre-specified data to be extracted (culture protocol, types and levels of interventions applied, method of detection and enumeration, and results) differed across studies. For studies that tested multiple types and levels of interventions, it was not feasible to report all information in the data extraction file given time and resource constraints. Determining which data to report that were representative of the overall findings was time-intensive. Conversely, some studies provided little information to be extracted, and identifying any information that would meet the data extraction protocol was difficult. Regarding reporting of results, some studies reported raw data or summaries of data, while other studies reported data in graph form. Where results were reported

in graph form, data was not extracted from the graphs, but overall trends in the findings were noted and every effort was made to identify quantitative measures in the text.

CHAPTER 3. EVALUATION OF STUDY QUALITY, RELEVANCE

INTRODUCTION

Aim 2. Evaluate studies identified for the post-harvest module based on study quality and relevance.

Post-harvest studies that were selected and underwent data extraction using systematic review methods in Chapter 2 were evaluated for quality and relevance in this chapter. Study quality and relevance criteria were developed using guidance from EFSA (2010). Given a limited number of studies available using systematic review methodology to assess the quality of evidence on food safety interventions, quality criteria were also based on the Bucher et al. (2012) risk of bias assessment and assessment of methodological soundness, to support a systematic review of chilling and processing interventions for *Salmonella* contamination in chicken.

EFSA (2010) defines methodological quality as characteristics of study design, execution, analysis and reporting that may cause a study to present a biased result, with findings at risk of deviating systematically from the truth. Methodological quality assessment components (EFSA 2010) were adapted for the purpose of this thesis, with indices of study quality characterized under execution, methodology, risk of bias, and analysis criteria. Study design characteristics fall under execution, and include use of graduated intervention levels, number of treatment groups, number of replicate trials, number of oysters per treatment group (if applicable), and whether validation to verify study methods and reported results was performed. Reporting completeness (reporting of methods and results for reproducibility) fell under the risk of bias assessment (Bucher et al. 2012). Use of controls and randomization were also captured by the risk of bias assessment (Bucher et al. 2012). Analysis criteria include statistical modeling approach and classification of exposure and outcome (EFSA 2010). See Tables 20 and 21 for descriptions of the study quality evaluation criteria.

METHODS

Evaluation of study quality

Evaluation criteria were derived from similar evaluations in the FDA QMRA (2005) and modified with associated confidence ratings according to criteria developed by guidance (EFSA 2010, OHAT 2015a) and other systematic reviews (Bucher et al. 2012, Wikoff et al. 2017). Predefined criteria including study design, execution, analysis, reporting, and data strength, were used to evaluate study quality (Tables 20 and 21). Key criteria are highlighted in darker blue. Precision of enumeration criteria were developed from FAO/WHO (2016) and with input from subject matter expert Dr. Andy DePaola. Results from study quality evaluation are reported in heat map form, based on the use of heat maps in Wikoff et al. (2017) to display findings from Risk of Bias assessments.

Methodology criteria evaluated whether the study reported results of interventions quantitatively or qualitatively, enumeration methods used and their respective limits of detection, as well as consideration or control of interference from the food matrix (oyster meat) or microflora (other bacteria species). Methodology criteria were developed with input from Dr. Andy DePaola. Performance evaluation of enumeration methods were developed using FAO/WHO (2016) guidance on selection of methods to detect and enumerate *V. parahaemolyticus* in seafood. Incorporating the current state of the evidence on method performance and selection of methods appropriate to post-harvest interventions was critical to this thesis.

Study quality criteria were further classified as key criteria if they were determined to highly influence the overall quality of the study. Quantitative or qualitative results, precision of enumeration, use of controls, reporting of methods (reproducibility), and reporting of results were identified as key criteria based on guidance from Dr. Keeve Nachman and Dr. Ben Davis.

Existing guidance does not support the use of a scoring system that produces a numerical rating for each study, as scientific rationale for different weights accorded to each characteristic

of assessment of bias is often lacking and renders the justification and use of scores contentious (EFSA 2010). As such, confidence ratings were developed based on the confidence rating approach in Wikoff et al. (2017) and OHAT (2015). Study confidence ratings indicate the level of confidence in the evidence presented, based on study features (Wikoff et al. 2017). Each evaluation criteria were assigned a confidence rating of high (+), moderate (?), or low (-) (OHAT 2015a). OHAT (2015a) uses four descriptors to indicate the level of confidence, high, moderate, low, and very low; for the purposes of this thesis the low and very low confidence descriptors were combined.

Confidence ratings are also useful in identifying future research needs (OHAT 2015a) and align well with the PECO framework. High confidence suggests that future research is "very unlikely" to change confidence in the observed relationship between the intervention (type(s) and level of PHP applied) and outcome (log reduction of *V. parahaemolyticus* abundance) (OHAT 2015a). Alternatively, very low confidence indicates that future research is "very likely" to affect confidence in the apparent relationship between the intervention and the outcome (OHAT 2015a). Studies that received a high confidence rating for ("fully met") at least four of the key quality criteria, are considered high quality. Studies that received a high confidence rating for two of less of the key quality, are considered low quality according to this evaluation criteria.

Table 20. Study Quality Evaluation Criteria. Part 1.

Evaluation Criteria			Confidence Rating		
	Variable	**Description**	**(+)**	**?**	**(-)**
Execution	Graduated intervention levels		Yes - methods and results reported graduated levels	Yes - only methods report graduated levels	No
	# treatment groups	# of different PHP treatments tested	Reported, ≥ 2	Reported, < 2	Not reported
	# Replicate trials	Reported # of replicate trials / microbiological analyses	Yes	Referenced elsewhere	No
	# oysters per treatment group		≥ 12	$12 \geq n \geq 3$	Not reported
	Validation performed		Yes		No
Methodology	**Quantitative or qualitative**	Result / parameter reported quantitatively or qualitatively	Quantitative		Qualitative
	Precision of enumeration		•Selective enrichment such as 3-tube MPN (initial levels) or 5-tube MPN (processed samples) followed by PCR / LAMP / molecular methods •Direct plating probe-hybridization (for total Vp)	• Selective enrichment followed by direct plating •Conventional MPN •Direct plating probe-hybridization (pathogenic Vp)	•Molecular methods (PCR) without selective enrichment •Selective enrichment followed by direct plating on selective medium that cannot adequately recover stressed but viable cells (TCBS) without resuscitation step
	LOD	Reported LOD	Yes	Standard method, reported elsewhere	No

Table 21. Study Quality Evaluation Criteria. Part 2.

Evaluation Criteria			Confidence Rating		
	Variable	**Description**	**(+)**	**?**	**(-)**
Methodology	Interference	From matrix or microflora	Accounted for in methodology	Discussed	Not discussed or accounted for
Risk of bias	**Controls**		Yes		No
	Reporting of methods / reproducibility	Protocols reported sufficiently to be reproduced	Yes	Methods reported elsewhere	No
	Reporting of results	Raw or summarized data reported	Yes - data	Yes - figures only	No
	Randomization	Reported intervention assigned randomly	Yes		No
Analysis	Statistical modeling approach	Described adequately to be reproduced	Yes	Statistical tests used were reported but program not provided, or vice versa	No
	Classification of exposure and outcome	Are exposure and outcome clearly described	Both clearly reported	One clearly reported; some outcomes reported for a range of exposures	Limited reporting

Evaluation of study relevance

Study relevance was also evaluated. Relevance is defined as the extent to which the study contributes to regionalization of the QMRA; addresses the assumptions used in the FDA 2005 QMRA; and has the data strength to support changes to the structure of the exposure assessment, or informs parameters in the post-harvest models for growth, log reduction, or another input (Table 22).

Indices of study relevance are region of data collection, analysis, and data strength to support s structural effect and / or parameter impact in the post-harvest model (Table 21). To support regionalization of the QMRA, studies that were conducted observationally in or collected samples from the Chesapeake Bay or Pacific Northwest met the relevance criteria for region. There was no distinction made in the relative importance of different relevance criteria. Studies that received a high relevance rating for ("fully met") at least two of the relevance criteria, are considered to have high relevance to the scope and purpose of the project.

Criteria for data strength to support a structural or parameter effect in the model were adapted from EFSA (2010). Data strength to support a parameter effect examines how the magnitude, direction, and uncertainty of the parameter, such as growth rate or cooldown rate, may directly impact the model for abundance of *V. parahaemolyticus* per g of raw oyster at retail. Data strength to support a structural effect in the post-harvest module of the exposure assessment examines how the evidence supports the addition, removal, or change in position of a parameter (such as reduction rate, see Figure 4) in the post-harvest supply chain. Each criterion was assigned a relevance rating of high (+), moderate (?), or low (-).

Table 22. Study Relevance Criteria.

Relevance Criteria			Relevance Rating		
Study Relevance	**Variable**	**Description**	**(+)**	**?**	**(-)**
Study Variable	Region	Region samples collected from (harvest, post-harvest observational studies)	WA state (Pacific NW) and/ or Chesapeake Bay		Neither WA state or CB
Analysis	Assumptions testing	Study challenges assumptions underlying FDA 2005 QMRA	Yes	Partial	No
Data strength to support:	Structural effect in model	Findings anticipated to change the exposure assessment diagram (add, remove, or change position of parameter)	Yes	Potentially	No
	Parameter impact in model	Findings anticipated to affect the magnitude and direction of the direct impact of parameter in model; sensitivity analysis, input with uncertainty distribution	Yes	Potentially	No

RESULTS

Evaluation of study quality:

Results for study quality evaluation are presented in heat map form (Tables 23 - 34) based on Wikoff et al. (2017), separately for each PHP or for a group of similar PHPs. Green indicates a high (+) confidence rating, yellow indicates a moderate (?) confidence rating, and red indicates a low (-) confidence rating. If a study receives a high confidence rating for a study quality criterion, it is interpreted to have fully met that criterion. Likewise, a moderate confidence rating indicates the study has partially met that criterion, and a low confidence rating indicates the study has not met the criterion.

Study quality results focused on acid PHPs are summarized in Table 23. Of the 10 studies on acid stress or adaptation, none fully met all 5 key quality criteria. 6 studies: Chiang 2008a, Chiang 2009, Chiang 2014, Lin 2004, Lin 2013, and Yeung 2004 fully met 4 key criteria and partially met precision of enumeration criteria. Moderate confidence in precision of enumeration was a common limitation of acid studies (Table 23).

Study quality results focused on cold storage are summarized in Tables 24 and 25. Of the 24 studies on cold and/or frozen storage, none fully met all 5 key quality criteria. Chiang 2006, Jones 2017, Lin 2004, Wu 2007, Ye 2013, and Zarei 2014 fully met 4 key criteria and partially met precision of enumeration criteria. DePaola 2009, DePaola 2010, and Liao 2017 fully met 4 key criteria but lacked controls. Fernandez-Piquer 2011 fully met 3 key criteria, and partially met precision of enumeration and reporting of results criteria. Burnham 2009, Drake 2008, Fernandez-Piquer 2010, Parveen 2013, and Shen 2009 fully met 3 key criteria, partially met precision of enumeration criteria, and lacked controls. The most common limitations for the key criteria were moderate or low confidence in precision of enumeration, and moderate confidence in reporting of results. For all remaining quality criteria, a common deficiency was that there was no performance of validation.

Study quality results focused on depuration are summarized in Table 26. Of the 12 depuration studies, only Yu 2010 fully met all 5 key quality criteria. Aagesen 2014, Ramos 2012 and Ming 2018 fully met 4 key criteria and partially met precision of enumeration criteria. Aagesen 2013, Su 2010, Phuvasate 2012, and Larsen 2015 fully met 3 key criteria, partially met precision of enumeration criteria, and lacked controls. Of note, no studies on depuration performed validation of the methods, and less than half of studies applied graduated levels of the intervention (Table 26). Study quality results focused on disinfectants are summarized in Tables 27 and 28. Of the 15 studies on disinfectants, none fully met all key quality criteria. Chiang 2006, Chiang 2008a, Chiang 2009, Chiang 2014, Chiu 2006, Lin 2004, Lin 2013, Shi 2017, Wang 2010a and Zarei 2014 fully met 4 key criteria, and partially met precision criteria. Takahashi 2016 fully met 3 key criteria, partially met precision of enumeration criteria, and lacked controls. Park 2018 had controls for sensory evaluation of PHP treated oysters and fully met 3 key criteria but did not meet precision of enumeration criteria. Common quality deficiencies of disinfectant studies were randomization, interference, and validation.

Study quality results focused on HHP are summarized in Table 29. Of the 13 studies investigating HHP, only Ma 2011 fully met all 5 key quality criteria. Ye 2011, Ye 2012, and Ye 2013 fully met 4 key criteria, and partially met precision of enumeration criteria. DePaola 2009 fully met 4 key criteria but lacked controls. Chen 2006, Chen 2007, and Hu 2005 fully met 3 key criteria, partially met precision of enumeration criteria, and lacked controls. Common deficiencies were controls, randomization, and validation (Table 29). Study quality results focused on icing are summarized in Table 30. Of the 4 studies on icing, Lydon 2015 and Thomas 2016 fully met all 5 key quality criteria, and Jones 2017 fully met 4 key criteria. Melody 2008a did not meet precision of enumeration criteria and had partial reporting of results.

Study quality results focused on irradiation are summarized in Table 31. Of the 9 studies on irradiation (gamma, X-ray, UV, LED) and the study on low-amperage electric current (Jin 2009), none fully met all 5 key quality criteria. However, 3 studies: Mahmoud 2009 (oyster

homogenate and culture experiments), Hou 2016, and Thulipa 2011, fully met 4 key criteria and partially met precision of enumeration criteria. Hu 2005 fully met 3 key criteria, partially met precision of enumeration criteria, and lacked controls. Common deficiencies for these studies were validation, randomization, and a limit of detection. Study quality results that focused on other interventions such as D-Tryophan, low salinity, growth in the food matrix and under different temperature, salinity, and pH conditions are summarized in Table 32. For the study on D-Tryptophan, Chen 2018 fully met 4 key quality, and partially met precision of enumeration criteria. Similar to other types of PHPs, validation was not performed. Huang 2012 and Wang 2018b both fully met four key quality criteria, with precision of enumeration a common limitation.

Study quality results focused on relaying PHPs are summarized in Table 33. Of the 7 studies on salinity relay, Parveen et al. 2017 and Yu et al. 2010 met all 5 key criteria. Taylor 2018 met 4 key criteria but lacked a control treatment group. Common deficiencies were graduated treatment levels, validation, controls, and randomization. For the study quality evaluation of the 3 studies on re-submersion and intertidal exposure, all studies on re-submersion (Kinsey 2015, Grodeska 2017) and intertidal exposure (Jones 2016) met the 5 key criteria for study quality (Table 33). However, none of the studies specified a limit of detection (Table 34).

Study quality results focused on temperature shock or stress are summarized in Tables 35 and 36. None of the 14 total studies fully met all 5 key criteria for study quality. 6 studies fully met 4 key criteria, with precision of enumeration partially met: Aagesen et al. 2014, Chiang 2006, Chiang 2009, Chiang 2014, Lai 2013, and Zarei 2014. DePaola 2009 fully met 4 key criteria but lacked controls. Whitaker 2010 fully met 3 key criteria, partially met precision of enumeration criteria, and lacked controls. Notably, no studies performed validation or applied randomization in treatment allocation. Precision of enumeration was a common limitation (Tables 35 and 36).

Table 23. Heat map of study quality evaluation results: Acid studies.

Study Confidence Rating	Wong 2004	Yeung 2004	Lin 2013	Hasegawa 2013	Chiang 2008a	Chiang 2014	Whitaker 2010	Chiang 2009	Kalburge 2014	Lin 2004
Execution										
Graduated interventions	+	+	+	-	+	+	+	-	+	+
# treatment groups	+	+	+	+	+	+	+	+	+	+
# Replicates	+	+	+	+	+	+	+	+	+	+
# oysters/ group	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Validation	-	-	-	-	-	-	-	-	-	-
Methodology										
Quantitative or qualitative	+	+	+	-	+	+	+	+	-	+
Precision of enumeration	?	?	?	?	?	?	?	?	?	?
LOD	-	+	-	+	-	-	+	-	+	-
Interference	+	-	-	-	-	-	-	-	+	-
Risk of Bias										
Controls	+	+	+	-	+	+	-	+	-	+
Reporting of methods	+	+	+	+	+	+	+	+	+	+
Reporting of results	?	+	+	?	+	+	+	+	?	+
Randomization	-	-	-	-	-	-	-	-	-	-
Analysis										
Statistical modeling	+	+	+	+	+	+	+	+	+	+
Classification of exposure and outcome	+	+	?	+	+	+	+	+	?	+

Table 24. Heat map of study quality evaluation results: Cold storage. Part 1.

Study Confidence Rating	Burnham 2009	Chang 2004	Drake 2008	Fernandez-Piquer 2011	Fernandez-Piquer 2010	Huang 2018	Liao 2017	Lin 2004	Mudoh 2010	Mudoh 2014	Parveen 2013	Jones 2017
Execution												
Graduated interventions	+	+	-	+	+	+	+	+	+	+	+	+
# treatments	+	+	+	+	+	+	+	+	+	+	+	+
# Replicate trials	+	+	+	+	-	+	+	+	+	+	-	?
# oysters per treatment	N/A	N/A	N/A	?	+	-	?	N/A	?	+	?	?
Validation	-	-	+	+	+	-	+	-	-	-	+	-
Methodology												
Quantitative or qualitative	+	-	+	+	+	+	+	+	+	+	+	+
Precision of enumeration	?	?	?	?	+	-	+	?	?	-	?	+
LOD	-	-	+	+	-	-	-	-	-	-	+	+
Interference	-	-	-	+	+	+	+	-	+	+	+	+
Risk of Bias												
Controls	-	+	-	+	-	-	-	+	-	-	-	-
Reporting of methods	+	+	+	+	+	+	+	+	-	+	+	+
Reporting of results	+	+	+	?	?	+	+	+	+	+	+	+
Randomization	-	-	-	-	-	+	+	-	+	+	-	-
Analysis												
Statistical modeling	+	+	+	+	+	+	+	+	+	+	+	+
Classify exposure, outcome	+	+	+	+	?	+	+	+	?	+	+	+

Table 25. Heat map of study quality evaluation results: Cold storage. Part 2.

Study Confidence Rating	Prapaiwong 2009	Vasudevan 2006	Wang 2018a	Ye 2013	Zarei 2014	Liu 2009	Shen 2009	Wu 2007	Chiang 2006	DePaola 2009	DePaola 2010	Songsaeng 2010
Execution												
Graduated interventions	+	+	+	+	+	+	+	-	+	-	-	-
# treatments	+	+	+	+	+	+	+	+	+	+	-	+
# Replicate trials	+	+	+	+	+	+	?	+	+	-	+	+
# oysters per treatment group	?	N/A	+	+	N/A	+	+	+	N/A	+	+	+
Validation	-	-	-	+	-	+	-	-	-	-	-	-
Methodology												
Quantitative or qualitative	-	+	+	+	+	+	+	+	+	+	+	-
Precision of enumeration	-	-	?	?	?	?	?	?	?	+	+	?
LOD	+	-	-	+	-	?	+	+	-	+	+	+
Interference	+	-	+	+	-	+	+	+	-	+	+	+
Risk of Bias												
Controls	+	-	-	+	+	+	-	-	+	-	-	-
Reporting of methods	+	+	+	+	+	+	+	+	+	+	+	+
Reporting of results	?	+	?	+	+	+	+	+	+	+	+	?
Randomization	+	-	-	-	-	+	-	+	-	-	-	-
Analysis												
Statistical modeling	+	+	-	+	+	+	+	+	+	+	+	+
Classification of exposure and outcome	+	+	+	+	+	+	+	+	+	+	?	+

Table 26. Heat map of study quality evaluation results: Depuration studies.

Study Confidence Rating	Aagesen 2013	Chae 2009	Su 2010	Sobrinho 2014	Phuvasate 2012	Yu 2010	Ramos 2012	Wang 2010b	Phuvasate 2013	Ming 2018	Larsen 2015	Aagesen 2014
Execution												
Graduated interventions	-	+	-	-	+	-	+	-	+	-	+	-
# treatments	+	+	+	+	+	+	+	+	+	+	+	+
#Replicate trials	+	-	+	-	-	?	+	+	+	-	+	+
# oysters per treatment group	+	+	?	+	+	+	+	+	+	+	+	+
Validation	-	-	-	-	-	-	-	-	-	-	-	-
Methodology												
Quantitative or qualitative	+	+	+	+	+	+	+	+	+	+	+	+
Precision of enumeration	?	-	?	-	?	+	?	-	-	?	?	?
LOD	-	+	-	-	+	-	+	-	-	+	+	-
Interference	+	+	+		+	+	+	+	+	+	+	+
Risk of Bias												
Controls	-	-	-	-	-	+	+	+	-	+	-	+
Reporting of methods	+	+	+	-	+	+	+	+	+	+	+	+
Reporting of results	+	+	+	+	+	+	+	+	+	+	+	+
Randomization	-	+	+	-	+	-	+	-	-	-	-	-
Analysis												
Statistical modeling	+	+	+	+	+	-	+	+	+	+	+	+
Classification of exposure and outcome	+	+	+	?	+	+	+	+	+	+	+	+

Table 27. Heat map of study quality evaluation results: Disinfectant studies. Part 1.

Study Confidence Rating	Chiang 2006	Chiang 2008a	Chiang 2008b	Chiang 2014	Chang 2004	Lai 2013	Park 2018	Takahashi 2016
Execution								
Graduated interventions	+	+	-	+	+	+	+	+
# treatments	+	+	+	+	+	+	+	+
# Replicates	+	+	+	+	+	+	+	-
# oysters per treatment group	N/A	N/A	N/A	N/A	N/A	N/A	-	N/A
Validation	-	-	-	-	-	-	-	-
Methodology								
Quantitative or qualitative	+	+	-	+	-	+	+	+
Precision of enumeration	?	?	?	?	?	?	-	?
LOD	-	-	+	-	-	-	+	-
Interference	-	-	-	-	-	-	+	-
Risk of Bias								
Controls	+	+	+	+	+	+	?	-
Reporting of methods	+	+	+	+	+	+	+	+
Reporting of results	+	+	?	+	+	?	+	+
Randomization	-	-	-	-	-	-	+	-
Analysis								
Statistical modeling	+	+	+	+	+	+	+	+
Classification of exposure and outcome	+	+	?	+	+	?	+	+

Table 28. Heat map of study quality evaluation results: Disinfectant studies. Part 2.

Study Confidence Rating	Chiu 2006	Chiang 2009	Lin 2004	Lin 2013	Zarei 2014	Wang 2010a	Shi 2017
Execution							
Graduated interventions	+	-	+	+	+	+	+
# treatments	+	+	+	+	+	+	+
# Replicates	+	+	+	+	+	+	+
# oysters per treatment group	N/A	N/A	N/A	N/A	N/A	+	N/A
Validation	-	-	-	-	-	-	?
Methodology							
Quantitative or qualitative	+	+	+	+	+	+	+
Precision of enumeration	?	?	?	?	?	-	?
LOD	+	-	-	-	-	-	-
Interference	?	-	-	-	-	+	-
Risk of Bias							
Controls	+	+	+	+	+	+	+
Reporting of methods	+	+	+	+	+	+	+
Reporting of results	+	+	+	+	+	+	+
Randomization	-	-	-	-	-	+	-
Analysis							
Statistical modeling	+	+	+	+	+	?	+
Classification of exposure and outcome	+	+	+	?	+	+	+

Table 29. Heat map of study quality evaluation results: High Pressure studies.

Study Confidence Rating	Chen 2006	DePaola 2009	Ye 2013	Ye 2012	Ma 2011	Kural 2008	Ye 2011	Koo 2006	Prapaiwong 2009	Phuvasate 2015	Chen 2007	Hu 2005	Vu 2018
Execution													
Graduated interventions	+	-	+	+	+	+	+	+	+	+	+	+	+
# treatment groups	+	+	+	+	+	+	+	+	+	+	+	+	+
# Replicates	+	-	+	+	+	+	+	+	+	+	+	+	-
# oysters/ group	N/A	+	+	+	+	?	?	?	?	?	N/A	?	N/A
Validation	-	-	+	-	+	-	-	-	-	-	-	-	-
Methodology													
Quantitative or qualitative	+	+	+	+	+	+	+	+	-	+	+	+	+
Precision of enumeration	?	+	?	?	+	-	?	-	-	-	?	?	-
LOD	-	+	+	+	+	-	+	+	+	+	-	-	+
Interference	-	+	+	+	+	+	+	+	+	+	-	+	-
Risk of Bias													
Controls	-	-	+	+	+	-	+	+	+	-	-	-	-
Reporting of methods	+	+	+	+	+	+	+	+	+	+	+	+	+
Reporting of results	+	+	+	+	+	+	+	+	?	+	+	+	+
Randomization	-	-	-	+	+	-	+	-	+	-	-	-	-
Analysis													
Statistical modeling	+	+	+	+	+	+	+	-	+	+	+	+	+
Classification of exposure and outcome	?	+	+	+	+	+	+	+	+	–	+	+	+

Table 30. Heat map of study quality evaluation results: Icing studies.

Study Confidence Rating	Melody 2008a	Lydon 2015	Thomas 2016	Jones 2017
Execution				
Graduated intervention levels	-	-	-	+
# treatment groups	+	+	+	+
# Replicate trials	+	+	+	?
# oysters per treatment group	+	+	+	?
Validation performed	-	-	-	-
Methodology				
Quantitative or qualitative	+	+	+	+
Precision of enumeration	-	+	+	+
LOD	-	+	-	+
Interference	+	+	+	+
Risk of Bias				
Controls	+	+	+	-
Reporting of methods	+	+	+	+
Reporting of results	?	+	+	+
Randomization	+	+	-	-
Analysis				
Statistical modeling approach	+	+	-	+
Classification of exposure and outcome	+	+	+	+

Table 31. Heat map of study quality evaluation results: Irradiation and electric current.

Study Confidence Rating	Abdallah 2009	Mahmoud 2009	Mahmoud 2009	Hu 2005	Park 2018	Yagi 2007	Nakahashi 2014	Hou 2016	Thupila 2011	Andrews 2011	Jin 2009
Execution		Oyster	Culture								
Graduated interventions	+	+	+	+	+	-	+	-	+	-	+
# treatment groups	+	+	+	+	+	?	+	+	+	+	+
# Replicates	-	+	+	+	+	+	+	+	+	+	+
# oysters/ group	N/A	-	N/A	?	-	N/A	N/A	N/A	-	+	N/A
Validation	-	-	-	-	-	-	-	-	-	-	-
Methodology											
Quantitative or qualitative	-	+	+	+	+	+	+	+	+	+	-
Precision of enumeration	+	?	?	?	-	?	?	?	?	-	?
LOD	-	+	+	-	+	-	-	-	-	-	+
Interference	-	+	+	+	+	-	-	-	+	+	-
Risk of Bias											
Controls	+	+	+	-	?	+	-	+	+	-	-
Reporting of methods	+	+	+	+	+	-	-	+	+	+	?
Reporting of results	?	+	+	+	+	?	+	+	+	?	?
Randomization	-	-	-	-	+	-	-	-	+	-	-
Analysis											
Statistical modeling	-	+	+	+	+	?	+	+	+	-	-
Classification of exposure and outcome	?	+	+	+	+	?	+	+	+	?	+

Table 32. Heat map of study quality evaluation results: Other studies.

Study Confidence Rating	Chen 2018	Huang 2012	Wang 2018b	Fujikawa 2009
Execution				
Graduated intervention levels	+	-	-	+
# treatment groups	+	+	+	+
# Replicate trials	+	+	+	-
# oysters per treatment group	?	N/A	?	N/A
Validation performed	-	-	-	-
Methodology				
Quantitative or qualitative	+	+	+	-
Precision of enumeration	?	?	-	?
LOD	+	-	-	-
Interference	+	-	+	-
Risk of Bias				
Controls	+	+	+	
Reporting of methods / reproducibility	+	+	+	+
Reporting of results	+	+	+	-
Randomization	+	-	-	-
Analysis				
Statistical modeling approach	+	+	+	+
Classification of exposure and outcome	+	+	+	?

Table 33. Heat map of study quality evaluation results: Relay studies.

Study Confidence Rating	Elmahdi 2018	Walton 2013	Parveen 2017	Melody 2008b	Yu 2010	Jahncke 2011	Taylor 2018
Execution							
Graduated intervention levels	-	-	-	-	-	+	-
# treatment groups	+	+	+	+	+	+	+
# Replicate trials	+	+	+	+	?	+	+
# oysters per treatment group	+	+	+	?	+	-	+
Validation performed	-	-	+	-	-	-	-
Methodology							
Quantitative or qualitative	+	+	+	+	+	+	+
Precision of enumeration	-	?	+	-	+	-	+
LOD	-	-	+	-	-	-	-
Interference	+	+	+	+	+	+	+
Risk of Bias							
Controls	-	-	+	-	+	-	-
Reporting of methods / reproducibility	+	?	+	+	+	?	+
Reporting of results	+	?	+	?	+	?	+
Randomization	-	+	+	-	-	-	-
Analysis							
Statistical modeling approach	+	?	+	+	-	-	+
Classification of exposure and outcome	+	+	+	+	+	+	+

Table 34. Heat map of study quality evaluation results: Re-submersion and intertidal studies.

Study Confidence Rating	Kinsey 2015	Grodeska 2017	Jones 2016
Execution			
Graduated intervention levels	+	+	-
# treatment groups	+	+	+
# Replicate trials	+	+	+
# oysters per treatment group	+	+	?
Validation performed	+	+	-
Methodology			
Quantitative or qualitative	+	+	+
Precision of enumeration	+	+	+
LOD	-	-	-
Interference	+	+	+
Risk of Bias			
Controls	+	+	+
Reporting of methods / reproducibility	+	+	+
Reporting of results	+	+	+
Randomization	-	+	-
Analysis			
Statistical modeling approach	+	+	+
Classification of exposure and outcome	+	+	+

Table 35. Heat map of study quality evaluation results: Temperature shock, stress studies. Part 1.

Study Confidence Rating	Aagesen 2014	Chiang 2009	DePaola 2009	Zarei 2014	Hasegawa 2013	Chiang 2006	Chiang 2008b
Execution							
Graduated intervention levels	-	-	-	+	-	+	-
# treatment groups	+	+	+	+	+	+	+
# Replicate trials	+	+	-	+	+	+	+
# oysters per treatment group	+	N/A	+	N/A	N/A	N/A	N/A
Validation performed	-	-	-	-	-	-	-
Methodology							
Quantitative or qualitative	+	+	+	+	-	+	-
Precision of enumeration	?	?	+	?	?	?	?
LOD	-	-	+	-	+	-	+
Interference	+	-	+	-	-	-	-
Risk of Bias							
Controls	+	+	-	+	-	+	+
Reporting of methods / reproducibility	+	+	+	+	+	+	+
Reporting of results	+	+	+	+	?	+	?
Randomization	-	-	-	-	-	-	-
Analysis							
Statistical modeling approach	+	+	+	+	+	+	+
Classification of exposure and outcome	+	+	+	+	+	+	?

Table 36. Heat map of study quality evaluation results: Temperature shock, stress studies. Part 2.

Study Confidence Rating	Lai 2013	Chiang 2014	Chang 2004	Whitaker 2010	Kalburge 2014	Nishina 2004	Kim 2012
Execution							
Graduated intervention levels	+	+	+	+	+	+	+
# treatment groups	+	+	+	+	+	+	+
# Replicate trials	+	+	+	+	+	-	+
# oysters per treatment group	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Validation performed	-	-	-	-	-	-	-
Methodology							
Quantitative or qualitative	+	+	-	+	-	-	+
Precision of enumeration	?	?	?	?	?	-	-
LOD	-	-	-	+	+	-	-
Interference	-	-	-	-	-	-	-
Risk of Bias							
Controls	+	+	+	-	-	-	-
Reporting of methods / reproducibility	+	+	+	+	+	?	+
Reporting of results	?	+	+	+	?	?	+
Randomization	-	-	-	-	-	-	-
Analysis							
Statistical modeling approach	+	+	+	+	+	+	+
Classification of exposure and outcome	?	+	+	+	?	?	+

Evaluation of study relevance

Evaluation of study relevance summary results are reported in Table 37 for all PHPs.

Table 37. Summary results for evaluation of study relevance.

		Study variable		Analysis	Data strength to support	
PHP Method	**N studies**	**Region (CB)[1]**	**Region (PNW)[2]**	**Assumptions testing**	**Structural effect in model**	**Parameter impact in model**
Acid	10	0	0	2 (+), 3 (?)	2 (+), 7 (?)	3 (+), 1 (?)
Cold storage	24	4	3	11 (+), 3 (?)	6 (+), 5 (?)	19 (+), 4 (?)
Depuration	12	0	6	7 (+), 2 (?)	5 (+), 2 (?)	7 (+), 4 (?)
Disinfectant	15	0	2	4 (+), 2 (?)	6 (+), 9 (?)	5 (+), 2 (?)
Other	4	0	0	2 (+)	2 (+)	2 (+)
Electric current	1	0	0	0	? (+)	0
HHP	13	0	1	1 (?)	11 (+), 2 (?)	12 (+), 1 (?)
Icing	4	0	0	4 (+)	1 (+) 1 (?)	3 (+)
Intertidal	1	0	1	1 (+)	1 (?)	1 (+)
Irradiation	9	0	0	2 (+)	7 (+), 2 (?)	5 (+), 2 (?)
Relay	7	3	0	1 (+), 1 (?)	2 (+), 2 (?)	1 (+), 5 (?)
Re-submersion	3	0	1	2 (+)	2 (+), 1 (?)	1 (+)
Temperature shock, stress (heat, cold)	14	0	1	6 (+), 3 (?)	3 (+), 8 (?)	5 (+), 3 (?)
Total (unique)	88					

[1]Chesapeake Bay

[2]Pacific Northwest

For the following tables (Tables 38 – 51), studies that were considered highly relevant fully meet (+) at least two of the study relevance criteria. Studies considered moderately relevant, fully meet at least one and partially meet (?) at least one relevance criteria.

For the study relevance evaluation of acid studies, Wong 2004 and Lin 2013 fully met two relevance criteria; Chiang 2009, Hasegawa 2013, and Lin 2004 fully met one and partially

met one relevance criteria (Table 38). As seen in Tables 39 and 40 for cold storage studies, Burnham 2009, DePaola 2010, Liu 2009, Mudoh 2010, Mudoh 2014, and Wu 2007 fully met at least 3 relevance criteria. Chang 2004, DePaola 2009, Drake 2008, Jones 2017, Liao 2017, Parveen 2013, Shen 2009, Ye 2013, and Zarei 2014, fully met at least 2 relevance criteria. Lin 2004 and Wang 2018a fully met one and partially met one relevance criteria (Tables 39 and 40). For depuration studies, Aagesen 2013, Phuvasate 2012, Phuvasate 2013, and Su 2010 fully met at least three relevance criteria (Table 41). Chae 2009, Ramos 2012, and Yu 2010 fully met at least 2 relevance criteria. Sobrinho 2010 and Wang 2010b fully met one and partially meet one relevance criteria (Table 41).

For study relevance evaluation of disinfectant studies, Chang 2004, Chiu 2006, Lin 2013, Park 2018, Shi 2017, and Zarei 2014 fully met at least 2 relevance criteria; Chiang 2008b and Chiang 2009 fully met one and partially met one relevance criteria (Tables 42 and 43). For HHP studies, Ma 2011 fully met 3 relevance criteria; 10 studies fully met two relevance criteria; Chen 2006 fully met one and partially met one relevance criteria. For icing studies, Melody 2008a fully met 3 relevance criteria and Jones 2017 and Lydon 2015 fully met 2 relevance criteria (Table 44). For Other PHPs, Fujikawa 2009 and Wang 2018b fully met two relevance criteria, while Chen 2018 and Huang 2012 only met one relevance criteria (Table 45).

For the study relevance evaluation of irradiation studies, Andrews 2011, Hu 2005, Park 2018, and Thulipa 2011 fully met at least 2 relevance criteria (Table 44). Hou 2016, Mahmoud 2009, Nakahasi 2014, and Yagi 2007 fully met one and partially met one relevance criteria (Table 44). For relay studies, Elmahdi 2018, Parveen 2017, and Yu 2010 fully met 2 relevance criteria; Jahnke 2011 fully met one and partially met one relevance criteria (Table 47). For the study relevance evaluation of other PHPs, Fujikawa 2009 and Wang 2018b fully met two relevance criteria and Chen 2018 and Huang 2012 fully met two relevance criteria (Table 46). For the study relevance evaluation of re-submersion studies, all re-submersion studies fully met at least two

relevance criteria, and Jones 2016 fully met three and partially met one relevance criteria (Table 49). For studies on temperature shock and stress, Aagesen 2014, Chang 2004, DePaola 2009, Kim 2012, Nishina 2004, and Zarei 2014 fully met at least two relevance criteria (Tables 50 and 51). Chiang 2008b, Chiang 2009, and Hasegawa 2013 fully met one and partially met one relevance criteria.

Table 38. Study Relevance Evaluation: Acid.

Study Relevance	Wong 2004	Yeung 2004	Lin 2013	Hasegawa 2013	Chiang 2008a	Chiang 2014	Whitaker 2010	Kalburge 2014	Lin 2004	Chiang 2009
Study Variable										
Region	-	-	-	-	-	-	-	-	-	-
Analysis										
Assumptions testing	-	?	?	+	-	-	-	?	-	+
Data strength to support:										
Structural effect	+	?	+	?	?	?	?	-	?	?
Parameter impact	+	-	+	-	-	?	-	-	+	-

Table 39. Study Relevance Evaluation: Cold Storage. Part 1.

Study Relevance	Burnham 2009	Drake 2008	Fernandez-Piquer 2011	Fernandez-Piquer 2010	Ye 2013	Shen 2009	Mudoh 2014	Parveen 2013	Mudoh 2010	Liu 2009	Lin 2004	Songsaeng 2010
Study Variable												
Region	-	PNW	-	-	-	-	CB	CB	CB	PNW	-	-
Analysis												
Assumptions testing	+	+	-	-	-	+	+	?	+	+	-	-
Data strength to support:												
Structural effect	+	?	-	-	+	-	-	-	-	+	?	-
Parameter impact	+	?	+	+	+	+	+	+	+	+	+	?

Table 40. Study Relevance Evaluation: Cold Storage. Part 2.

Study Relevance	Wu 2007	Wang 2018a	Liao 2017	Huang 2018	Prapaiwong 2009	Zarei 2014	Vasudevan 2006	Chang 2004	Chiang 2006	DePaola 2009	DePaola 2010	Jones 2017
Study Variable												
Region	-	-	-	-	-	-	-	-	-	-	PNW, CB	-
Analysis												
Assumptions testing	+	?	+	-	-	+	-	?	-	-	+	+
Data strength to support:												
Structural effect	+	-	-	-	?	?	-	+	?	+	-	-
Parameter impact	+	+	+	+	?	+	?	+	-	+	+	+

Table 41. Study Relevance Evaluation: Depuration.

Study Relevance	Aagesen 2013	Chae 2009	Su 2010	Sobrinho 2014	Phuvasate 2012	Yu 2010	Ramos 2012	Wang 2010b	Phuvasate 2013	Ming 2018	Larsen 2015	Aagesen 2014
Study Variable												
Region	PNW	-	PNW	-	PNW	-	-	-	PNW	PNW	-	PNW
Analysis												
Assumptions testing	+	-	+	+	+	-	-	+	+	+	?	?
Data strength to support:												
Structural effect	?	+	?	-	-	+	+	-	-	+	-	+
Parameter impact	+	+	+	?	+	+	+	?	+	?	-	?

Table 42. Study Relevance Evaluation: Disinfectants. Part 1.

Study Relevance	Chiang 2006	Chiang 2008a	Chiang 2008b	Chiang 2014	Chang 2004	Park 2018	Lai 2013	Shi 2017
Study Variable								
Region	-	-	-	-	-	-	-	PNW
Analysis								
Assumptions testing	-	-	+	-	?	-	-	+
Data strength:								
Structural effect	?	?	?	?	+	+	?	+
Parameter impact	-	-	?	?	+	+	-	-

Table 43. Study Relevance Evaluation: Disinfectants. Part 2.

Study Relevance	Takahashi 2016	Chiu 2006	Chiang 2009	Lin 2004	Lin 2013	Zarei 2014	Wang 2010a
Study Variable							
Region	-	PNW	-	-	-	-	-
Analysis							
Assumptions testing	-	-	+	-	?	+	-
Data strength:							
Structural effect	?	+	?	?	+	?	+
Parameter impact	-	-	-	+	+	+	?

Table 44. Study Relevance Evaluation: High Hydrostatic Pressure.

Study Relevance	Chen 2006	DePaola 2009	Ye 2013	Ye 2012	Ma 2011	Kural 2008	Ye 2011	Koo 2006	Prapaiwong 2009	Phuvasate 2015	Chen 2007	Hu 2005	Vu 2018
Study Variable													
Region	-	-	-	-	PNW	-	-	-	-	-	-	-	-
Analysis													
Assumptions testing	-	-	-	-	-	?	-	-	-	-	-	-	-
Data strength to support:													
Structural effect	?	+	+	+	+	+	+	+	?	+	+	+	+
Parameter impact	+	+	+	+	+	+	+	+	?	+	+	+	+

Table 45. Study Relevance Evaluation: Icing.

Study Relevance	Thomas 2016	Melody 2008a	Lydon 2015	Jones 2017
Study Variable				
Region	-	-	-	-
Analysis				
Assumptions testing	+	+	+	+
Data strength to support:				
Structural effect	-	+	?	-
Parameter impact	-	+	+	+

Table 46. Study Relevance Evaluation: Other.

Study Relevance	Chen 2018	Huang 2012	Fujikawa 2009	Wang 2018b
Study Variable				
Region	-	-	-	-
Analysis				
Assumptions testing	-	-	+	+
Data strength to support:				
Structural effect in model	+	+	-	-
Parameter impact in model	-	-	+	+

Table 47. Study Relevance Evaluation: Irradiation and electric current.

Study Relevance	Abdallah 2009	Mahmoud 2009	Hu 2005	Park 2018	Yagi 2007	Nakahashi 2014	Hou 2016	Thupila 2011	Andrews 2011	Jin 2009
Study Variable										
Region	-	-	-	-	-	-	-	-	-	-
Analysis										
Assumptions testing	+	+	-	-	-	-	-	-	-	-
Data strength to support:										
Structural effect	?	?	+	+	+	+	+	+	+	?
Parameter impact	-	+	+	+	?	-	?	+	+	-

Table 48. Study Relevance Evaluation: Relay.

Study Relevance	Elmahdi 2018	Walton 2013	Parveen 2017	Melody 2008b	Yu 2010	Jahncke 2011	Taylor 2018
Study Variable							
Region	CB	-	CB	-	-	CB	-
Analysis							
Assumptions testing	+	-	-	-	-	-	?
Data strength to support:							
Structural effect	-	-	+	-	+	?	?
Parameter impact	?	-	?	?	+	?	?

Table 49. Study Relevance Evaluation: Re-submersion.

Study Relevance	Kinsey 2015	Jones 2016	Grodeska 2017
Study Variable			
Region	-	PNW	-
Analysis			
Assumptions testing	+	+	-
Data strength to support:			
Structural effect	+	?	+
Parameter impact	-	+	-

Table 50. Study Relevance Evaluation: Temperature shock, stress. Part 1.

Study Relevance	Aagesen 2014	Chiang 2009	DePaola 2009	Zarei 2014	Hasegawa 2013	Chiang 2006	Chiang 2008b
Study Variable							
Region	PNW	-	-	-	-	-	-
Analysis							
Assumptions testing	?	+	-	+	+	-	+
Data strength to support:							
Structural effect	+	?	+	?	?	?	?
Parameter impact	?	-	+	+	-	-	?

Table 51. Study Relevance Evaluation: Temperature shock, stress. Part 2.

Study Relevance	Lai 2013	Chiang 2014	Chang 2004	Whitaker 2010	Kalburge 2014	Kim 2012	Nishina 2004
Study Variable							
Region	-	-	-	-	-	-	-
Analysis							
Assumptions testing	-	-	?	-	?	+	+
Data strength to support:							
Structural effect	?	?	+	?	-	-	-
Parameter impact	-	?	+	-	-	+	+

DISCUSSION

Overview

Study quality and relevance were evaluated according to predefined criteria based on EFSA (2010) and OHAT (2015a, 2015b) guidance, as well as previous systematic reviews (Bucher et al. 2012, Wikoff et al. 2017). Overall quality and relevance of the state of the evidence available on PHPs to reduce *V. parahaemolyticus* in raw oysters was assessed to support a future QMRA for oysters harvested in the Chesapeake Bay and Washington state.

Study quality evaluation criteria with associated confidence ratings were used to classify all studies and identify high quality studies. Studies that are high quality have fully met (meaning, they received a high confidence rating for) 4 key quality criteria. For high quality studies, future research is not considered likely to change confidence in the apparent relationship between the intervention, the PHP method, and the outcome, log reduction (or other measure of effect) in *V. parahaemolyticus* abundance (OHAT 2015a). Studies that are low quality fully meet 2 or less key quality criteria, indicating that the study received high confidence ratings for less than half of the key quality criteria. Studies that are low quality most often lacked controls, were qualitative, and rated as low or moderate confidence in precision of enumeration methods used. For low quality studies, future research is more likely to change the confidence in the relationship between the intervention and the outcome. Future research should be prioritized to greater understand the effectiveness of those PHPs for which few or no high quality studies exist.

Study relevance criteria with associated relevance ratings were used to classify studies as high, medium, or low relevance. Studies that are moderately relevant fully meet (+) one relevance criteria and at least partially meet (?) another relevance criteria. Studies most commonly did not meet regional relevance criteria, followed by assumptions testing criteria. Unlike the Harvest Module, studies were not excluded from the Post-Harvest Module based on region. Studies that examine the effectiveness of a PHP treatment and factors that influence the parameter effect were largely conducted in controlled experimental settings, whose findings were considered

generalizable to the Chesapeake Bay and Pacific Northwest. For observational studies on depuration and relaying, the region where the study was conducted is more important, but studies conducted in other regions may still be informative. Studies that did not directly test assumptions used by the existing FDA QMRA may still contain the data strength to support structural or parameter effects in the post-harvest model.

Studies provided the data strength to support an anticipated parameter impact in the model if the study measured the magnitude, direction, and uncertainty of a parameter, such as growth rate during intertidal harvest or post-harvest (Figure 2), that was present in the post-harvest model of the existing FDA QMRA. Studies provided the data strength to support an anticipated structural effect in the post-harvest model if the study provided data on a parameter that was not directly included in the post-harvest model (such as, reduction rate from PHP applied, growth rate during storage, and growth or inactivation rate during cooldown) or the study measured data that may support the addition, removal, or change in position of a parameter in the post-harvest supply chain (Figure 4).

Post-Harvest Practices

Acid: There are an emerging number of studies on the effects of acid adaptation and acid stress in promoting or inhibiting growth of *V. parahaemolyticus*. Findings may suggest structural changes to the post-harvest module of the existing exposure assessment (Figure 4), incorporating growth response to acid, a food preservative, as no parameter currently exists to represent how the model may be affected by input of a food preservative. Studies that assessed acid response that have the potential for a parameter effect in the model are those that concomitantly examined *V. parahaemolyticus* response to heat shock and/ or cold shock. While 2 studies were considered of high relevance, 4 studies out of the total 10 studies on acid stress and/or acid adaptation were high quality, fully meeting four key criteria and partially meeting precision criteria.

Cold storage: Many studies looked at growth of *V. parahaemolyticus* during cold storage, quantifying the efficacy of different types of storage treatments (such as wet vs. dry) at a variety

of temperatures and durations to control population abundance. All studies appear to have some potential to have a parameter effect in an updated QMRA model, in that they may provide data that can better inform parameters in the existing post-harvest model. Studies that assessed growth during cold storage following application of a PHP may have a structural effect on the updated model, as no parameter was included in the original QMRA to quantify growth during cold storage, as determined by the inputs of cooldown duration, method used, and air temperature (Figure 4). Nine studies out of the identified 24 studies on cold storage were high quality, fully meeting four key criteria. While there were a relatively large number of studies on cold storage, less than half met sufficient criteria to be rated as high quality. Six studies were considered moderate quality, fully meeting at least 3 key criteria. Use of a weighted approach, weighting high quality studies more than moderate quality studies in model development and parameterization, would increase information available to be incorporated while still anchoring the model more heavily on findings observed in more rigorous and reliable studies.

Depuration: Studies on depuration can help to inform the distribution of log reduction achieved under different environmental conditions. A minority of depuration studies support a structural effect in the existing QMRA model to add depuration as an effective PHP. Many of the studies on depuration addressed assumptions used in the FDA QMRA, such as the assumption that water activity of oysters does not vary substantially. Water and purging activity of oysters was examined with changing temperature, feeding status, and type and size of oysters (Table A11). Four studies out of the 12 identified for depuration were high quality, and four studies were moderate quality; all partially met precision criteria, suggesting that methods of detection and enumeration typically used are limiting. As more of the depuration studies tended to be observational in design, lack of controls was a major reason studies did not meet more key quality criteria.

Disinfectants: An increasing number of studies examine the use of disinfectants in controlling the risk of cross-contamination on surfaces in the retail setting. These studies may

suggest a structural effect in the model to consider disinfectant use as a hazard control point for surfaces or oysters. Due to limited time and resources, three studies on electrolyzed oxidizing water were considered as potentially relevant but not included for full-text review. Future research may examine those studies to determine if electrolyzed oxidizing water, a disinfectant and sanitizer, poses potential for use in food processing stages of the post-harvest module. A majority of studies on disinfectants were high quality; of 15 total studies, 10 fully met 4 key quality criteria.

High hydrostatic pressure (HHP): Studies on HHP as a post-harvest practice overwhelmingly suggest structural changes to the existing model by adding a parameter for log reduction achieved by the PHP method inputs. Though HHP studies did not largely address assumptions used in the FDA QMRA, evidence indicates that HHP is an emerging and effective PHP. Four out of the 13 studies on HHP were high quality, and 3 studies were of moderate quality. Like depuration, lack of controls was a primary reason several studies did not meet more key quality criteria.

Icing: The few studies that tested icing, such as on board or at dockside, may update the existing QMRA with additional log reduction values. All 4 studies challenged assumptions from the FDA QMRA, but only one study (Melody 2008a) had the data strength to support a structural *and* parameter effect; only Thomas 2016 did not have the data strength to support a parameter impact. None of the icing studies were conducted in the Chesapeake Bay or Washington state. Three studies were considered high quality based on the key criteria.

Irradiation and electric current: Additional methods of post-harvest processing to control *V. parahaemolyticus* growth, such as irradiation using gamma rays, X-rays, UV-LED, or electric currents, are also emerging as effective methods to reduce *V. parahaemolyticus* growth while preserving oyster texture and other sensory quality attributes. Data suggests structural and parameter effects for irradiation log reduction in the post-harvest model. Three of 9 studies on irradiation were high quality, and one study was moderate quality. Consistent with the other

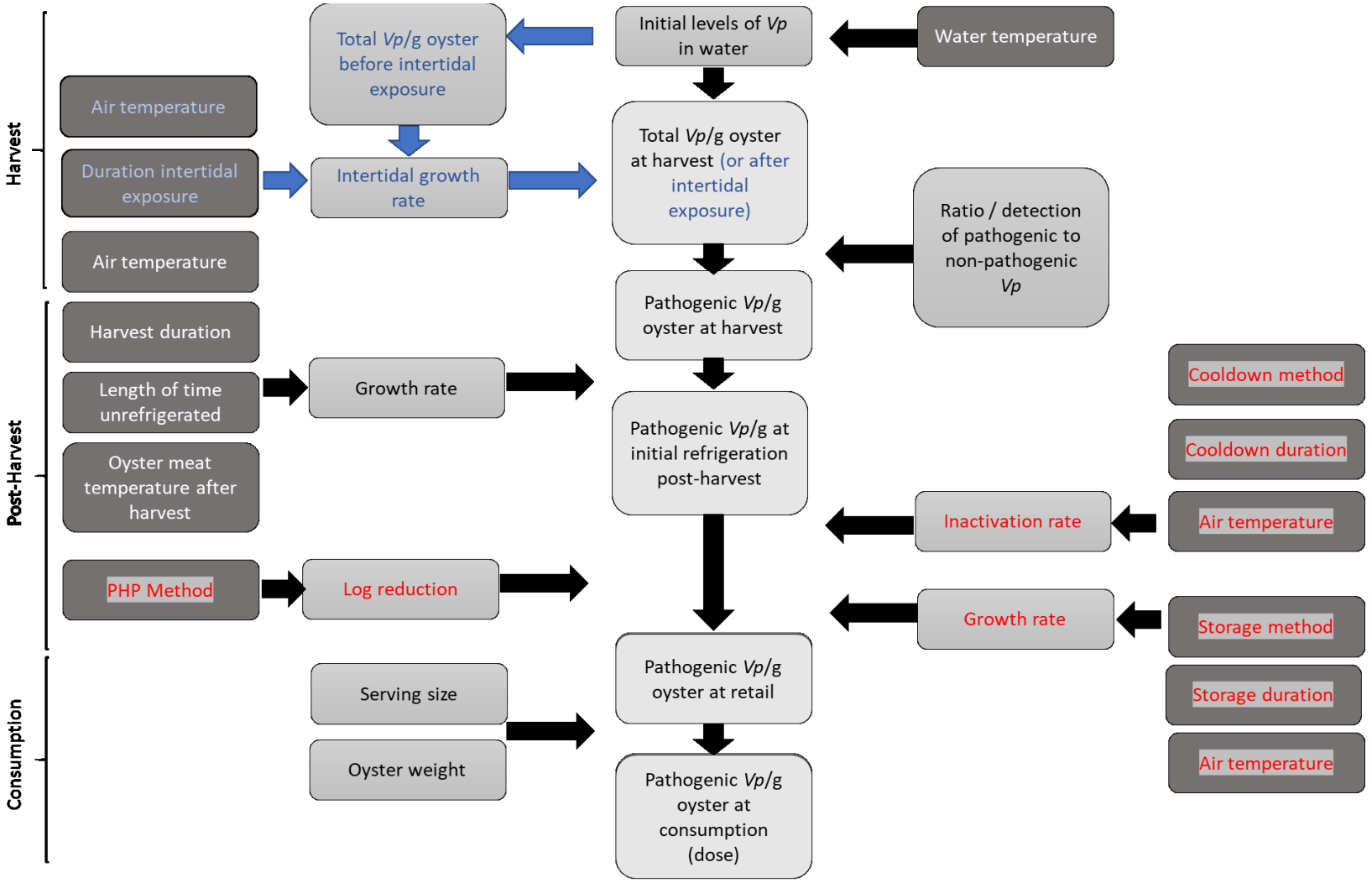
PHPs, no studies fully met precision criteria, highlighting detection and enumeration methods used as a consistent limitation.

Relay: Of the 7 high-salinity relaying studies included, 3 were conducted in the Chesapeake Bay, representing high relevance for informing regionalization of the QMRA. One study assessed intertidal exposure in the Pacific Northwest, highly relevant to a regional post-harvest model for Washington. However, there is limited data to suggest potential effects to model structure or parameters. Two relaying studies met all 5 key quality criteria, and one study was also high quality but lacked controls.

Re-submersion: A limited number of studies focused on re-submersion; while over half of the studies indicate potential changes to the post-harvest structure to include the effect of this PHP on *V. parahaemolyticus* abundance, most do not contribute parameter changes to the model. This is because the studies did not tend to inform existing parameters or inputs, such as post-harvest growth rate as determined by harvest duration, air temperature, length of time unrefrigerated, or oyster meat temperature after harvest (Figure 4). All 3 studies on re-submersion and intertidal exposure met the 5 key quality criteria.

Temperature shock or stress: Finally, 14 studies applied cold or heat shock and/ or temperature stress conditions to determine effect on *V. parahaemolyticus* growth. Such studies have implications for growth during intertidal exposure, and before initial refrigeration. A majority of those studies support a potential effect to model structure to update *V. parahaemolyticus* population dynamics during extreme heat or cold exposures. Of the 14 studies, seven were high quality and one was moderate quality, and applied heat or cold shock or stress in combination with other PHPs. This indicates that a majority of temperature shock or stress studies were high quality.

Figure 4. Suggested changes to the Exposure Assessment schematic. Post-harvest module suggested changes are in red. Dark grey boxes indicate input values; medium grey boxes indicate parameter values; and light grey boxes indicate abundance of Vp at stages of the supply chain.



CHAPTER 4. SYNTHESIS OF RESULTS, CONCLUSIONS

INTRODUCTION

A weight of evidence approach was used to synthesis results from the systematic review conducted in Aims 1 and 2 (Chapters 2 and 3) and to form conclusions about the strength and limitations of the information available. Those findings will be used in this chapter to propose recommendations regarding the efficacy of PHPs from the studies evaluated.

Systematic review methods used in this thesis are consistent with methods used by other systematic reviews for food safety risk assessments. Further, this thesis demonstrates how systematic review can enhance the transparency of the data selection process for QMRA models, as opposed to traditional narrative review methods. During systematic review, the search strategy was predefined with guidance from an informationist and applied to multiple databases to maximize literature captured by the search strategy. Using the PICO statement, objective inclusion and exclusion criteria were applied during screening to identify potentially relevant studies. During eligibility, two independent reviewers determined studies to be included for full-text review. Data extraction during full-text review was performed by one reviewer, but decisions to exclude studies during full-text review were confirmed with a second reviewer. The above steps have allowed for a rigorous and systematic examination of the data in order to identify changes to the post-harvest model of the exposure assessment.

This thesis aims to assist shellfish food safety decision makers on the effectiveness of various PHPs and implementation strategies to reduce the risk of illness. For this thesis, a 3.52 log reduction based on NSSP (2017) guidance, was used to represent the reference standard for minimum reduction achieved by a PHP to be considered a validated and effective process for pathogen reduction. However, the existing FDA QMRA model found that interventions that cause at least 4.5 log reductions decrease *V. parahaemolyticus* abundance and probability of illness such that epidemiological surveillance is unlikely to identify cases of illness (FDA 2005).

METHODS

Study quality and study relevance were used to form the weight of evidence for effective post-harvest practices. Studies that were considered high quality fully met four key quality criteria, and studies that were considered highly relevant fully met two relevance criteria. Studies that met both were classified as high quality and high relevance. Studies considered moderate quality fully met at least 3 key quality criteria, and studies that are moderately relevant fully meet one relevance criteria and at least partially meet another relevance criteria. All other studies were considered of low quality or of low relevance.

RESULTS

Results from the cross-tabulation of study quality and relevance (Table 52) indicate that while many studies can be considered of high quality *or* high relevance, relatively few studies per PHP method are of *both* high quality *and* high relevance using the criteria defined previously (Table 53).

Table 52. Cross-tabulation of study quality and relevance evaluation results.

Quality			
+	Acid: Chiang 2008a, Chiang 2014, Yeung 2004 Cold storage: Chiang 2006 Disinfectant: Chiang 2006, Chiang 2008a, Chiang 2014 Icing: Thomas 2016 Other: Chen 2018, Huang 2012 Relay: Taylor 2018 Re-submersion: Grodeska 2017 Temperature shock or stress: Chiang 2006, Chiang 2014	Acid, disinfectant: Chiang 2009, Lin 2004 Cold storage: Lin 2004 Depuration: Wang 2010b Irradiation: Hou 2016 Temperature shock or stress: Chiang 2009	Acid: Lin 2013 Cold storage: DePaola 2009, DePaola 2010, Liao 2017, Liu 2009, Ye 2013, Zarei 2014, Jones 2017 Depuration: Ming 2018, Ramos 2012, Yu 2010 Disinfectant: Chiu 2006, Lin 2013, Wang 2010a, Zarei 2014, Shi 2017 HHP: DePaola 2009, Koo 2006, Ma 2011, Ye 2011, Ye 2012, Ye 2013 Icing: Lydon 2015, Jones 2017 Irradiation: Mahmoud 2009, Thulipa 2011 Relay: Parveen 2017, Yu 2010 Re-submersion: Jones 2016, Kinsey 2015 Temperature shock or stress: Aagesen 2014, DePaola 2009, Zarei 2014 Other: Wang 2018b
?	Acid: Whitaker 2010 Cold storage: Fernandez-Piquer 2010, Fernandez-Piquer 2011, Huang 2018, Songsaeng 2010, Vasudevan 2006 Depuration: Larsen 2015 Disinfectant: Takahashi 2016, Lai 2013 Temperature shock or stress: Lai 2013, Whitaker 2010	Disinfectant, temperature shock or stress: Chiang 2008b HHP: Chen 2006, Irradiation: Abdallah 2009	Acid: Wong 2004 Cold storage: Burnham 2009, Chang 2004, Drake 2008, Mudoh 2014, Parveen 2013, Shen 2009, Wu 2007 Depuration: Aagesen 2013, Chae 2009, Phuvasate 2012, Phuvasate 2013, Su 2010 Disinfectant: Park 2018 HHP: Chen 2007, Hu 2005, Kural 2008, Phuvasate 2015, Vu 2018 Icing: Melody 2008a Irradiation: Hu 2005 Relay: Elmahdi 2018 Temperature shock or stress: Chang 2004, Kim 2012
-	Cold storage, HHP: Prapaiwong 2009 Irradiation: Jin 2009, Nakahashi 2014 Relay: Melody 2008b, Walton 2013 Temperature shock or stress: Kalburge 2014	Cold storage: Wang 2018a Depuration: Sobrinho 2014 Irradiation: Yagi 2007 Relay: Jahnke 2011 Temperature shock or stress: Hasegawa 2013	Cold storage: Mudoh 2010 Irradiation: Andrews 2011 Other: Fujikawa 2009 Temperature shock or stress: Nishina 2004
	-	?	+

Relevance

Table 53. Results of study quality and relevance synthesis.

PHP Method	# of studies of high quality and relevance	Names of studies
Acid	1	Lin 2013
Cold storage	7	DePaola 2009, DePaola 2010, Liao 2017, Wu 2007, Ye 2013, Zarei 2014, Jones 2017
Depuration	3	Ming 2018, Ramos 2012, Yu 2010
Disinfectant	5	Chiu 2006, Lin 2013, Shi 2017, Wang 2010a, Zarei 2014
HHP	6	DePaola 2009, Koo 2006, Ma 2011, Ye 2011, Ye 2012, Ye 2013
Icing	2	Lydon 2015, Jones 2017
Irradiation, electric current	2	Mahmoud 2009, Thulipa 2011
Other	1	Wang 2018b
Relay	2	Parveen 2017, Yu 2010
Re-submersion	2	Jones 2016, Kinsey 2015
Temperature shock, stress	3	Aagesen 2014, DePaola 2009, Zarei 2014
Total Unique	26	

As the NSSP (2017) guidance requires a PHP to achieve 3.52 log reductions for oysters to be labeled as having undergone a validated pathogen reduction process, that is the reference against which log reductions observed in the high quality and high relevance studies are compared in this thesis. Studies identified above as high quality and high relevance report the following log reductions or survival associated with various PHP methods. Results are reported in Tables 54 – 63:

Acid

Table 54. Log reductions of high quality and high relevance studies: Acid.

Study	PHP Method	Log reduction (log CFU/ml)
Lin 2013	Acid adaptation, Cl disinfectant	1.20 to 4.95

Synthesis of evidence from only studies of high quality and high relevance for acid PHPs are reported in Table 54. This shows that acid adaptation with chlorine may reduce *V. parahaemolyticus* populations up to 4.95 logs, but as only one acid study was of high quality and high relevance, more research is needed.

Cold storage

Table 55. Log reductions of high quality and high relevance studies: Cold Storage.

Study	PHP Method	Log reduction (log CFU/ml)	Survival rate (%) or ratio
DePaola 2009	Freezing, frozen storage	Final density: < .04 – 3 to > 30 MPN /g (reduced to < LOD)	
DePaola 2010	Cold storage	Final density: ≤ 1 to > 10^5 MPN/g	
Jones 2017	Ambient storage then refrigeration	0 to 1.6 log MPN/g increase	
Liao 2017	Cold storage	0.213 to 2.109 log CFU/g	
Wu 2007	Cold storage	1.4 to 2.21	
Wu 2007	Ice water storage	1.3 to 2.09	
Ye 2013	HHP then cold storage	0 to > 2.7 log MPN/g	
Ye 2013	Cold storage then HHP	5.4 to 5.9 log MPN/g	
Zarei 2014	Cold storage		12.6% (total), 17.4% (*tdh*+)
Zarei 2014	Cold storage, Chlorine stress		1.1% (total), 1.2% (*tdh*+)

Synthesis of evidence from only studies of high quality and high relevance for cold storage PHPs are reported in Table 55. While cold storage alone does not have the potential to achieve the 3.52 log reduction as required by NSSP (2017) for labeling, 7-day frozen storage following high pressure can achieve up to 7 log reductions of *V. parahaemolyticus*, effectively eliminating risk of illness from *V. parahaemolyticus* (Ye 2013) (Table 55).

Depuration

Table 56. Log reductions of high quality and high relevance studies: Depuration.

Study	PHP Method	Log reduction
Ming 2018	Depuration; depuration with algae feeding	2.47 log MPN/g; 2.19
Ramos 2012	Depuration, UV light, Cl	2 to 3.1 log MPN/g
Yu 2010	Depuration and recirculation or flow-through, creek relay	0.96 to 2.84 log MPN/g

Synthesis of evidence from only studies of high quality and high relevance for depuration PHPs are reported in Table 56. Depuration is not shown to effectively achieve at least 3.52 log reductions of *V. parahaemolyticus*, even with the use of sterilization practices such as UV light or chlorine (Table 56). However, it can achieve up to 3 log reductions, so depuration may be useful in colder months where ambient levels of *V. parahaemolyticus* are lower.

Disinfectant

Table 57. Log reductions of high quality and high relevance studies: Disinfectants.

Study	PHP Method	Log reduction (log CFU/ml)	Survival rate (%)
Chiu 2006	Electrolyzed oxidizing water on surfaces	4.02 to > 5	
Lin 2013	Chlorine disinfectant	4.28 to 6.84	
Lin 2013	Chlorine disinfectant, heat shock	1.21 to 5.52	
Lin 2013	Chlorine disinfectant, cold shock	1.33 to 5.41	
Shi 2017	Saline solution	4.74 to 7.15 log CFU/cm2	
Wang 2010a	Chlorine dioxide	2.3 to 3.1 log CFU/g	
Zarei 2014	Sodium chloride		14.5 to 19.3%
Zarei 2014	Chlorine stress, sodium chloride		4 to 5.6%

Synthesis of evidence from only studies of high quality and high relevance for disinfectant PHPs are reported in Table 57. Disinfectants are shown to achieve greater than 5 log reductions in *V. parahaemolyticus*, highlighting the efficacy of chlorine and other disinfectant use in preventing cross-contamination of surfaces with *V. parahaemolyticus* during handling and processing (Table 57).

HHP

Table 58. Log reductions of high quality and high relevance studies: HHP.

Study	PHP Method	Log reduction[1]
DePaola 2009	HHP	.04 - > 5 log MPN/g
Koo 2006	HHP	4.3 to 5.7 log CFU/g
Ma 2011	HHP	2.7 to > 3.53 log MPN/g
Ye 2011	HHP, incubation temp.	4.2 to 6.7 log CFU/g
Ye 2012	HHP	1.6 to 7.8 log MPN/g
Ye 2012	HHP, mild heat	3.6 to < 7.6 log MPN/g
Ye 2013	HHP	3.9 to 6.5 log MPN/g
Ye 2013	Storage (varying temp.) then HHP	5.4 to 7.4 log MPN/g
Ye 2013	HHP then cold storage	0 to > 2.7 log MPN/g

Synthesis of evidence from only studies of high quality and high relevance HHP are reported in Table 58. Findings show that high pressure can achieve greater than 7 log reductions, independently or in concert with other post-harvest processes such as cold storage and mild heat. High pressure presents as an effective post-harvest practice that meets regulatory requirements and would reduce or eliminate risk of illness, particularly important in months with higher abundance of *V. parahaemolyticus.*

Icing

Table 59. Log reductions of high quality and high relevance studies: Icing.

Study	PHP Method	Increase	Log reduction
Jones 2017	Icing	0.8 log MPN/g	0.3 to 0.4 log MPN/g
Lydon 2015	Ice slurry	2.74 log MPN/g (total) .09 MPN/g (*tdh*+); 0.16 MPN/g (*trh*+)	

Synthesis of evidence from only studies of high quality and high relevance icing PHPs are reported in Table 59. Icing post-harvest, on board or dockside was not shown to effectively reduce *V. parahaemolyticus* density in oysters. In fact, *V. parahaemolyticus* remained unchanged or even increased in oysters subjected to icing, which negatively impacted oyster survival during subsequent cold storage. Currently, data do not support icing as a PHP to predictably reduce *V. parahaemolyticus*, particularly in warmer months.

Irradiation, Other

Table 60. Log reductions of high quality and high relevance studies: Irradiation and Other.

Study	PHP Method	Log reduction (log CFU/ml)
Mahmoud 2009	X-ray irradiation	1 to > 7 log MPN/g
Thulipa 2011	Irradiation	0.55 to 4.38 log CFU/g
Wang 2018b	Other (Food matrix)	5.91 to 6.51 (*tdh*+); 5.8 to 6.8 (*trh*+) log CFU/g

Synthesis of evidence from only studies of high quality and high relevance for irradiation and Other (food matrix) are reported in Table 60. Two irradiation studies were found to be of high quality and relevance, and suggest that irradiation can achieve 3.52 log reductions without negatively affecting oyster quality.

Relay

Table 61. Log reductions of high quality and high relevance studies: Relay.

Study	PHP Method	Log reduction (log CFU/ml)
Parveen 2017	High salinity relay	1 to > 2 log MPN/g
Yu 2010	High salinity relay	1.57 to 2.84 log MPN/g

Synthesis of evidence from only studies of high quality and high relevance for relaying PHPs are reported in Table 61. High salinity relay achieved some measure of log reduction in *V. parahaemolyticus*, but it does meet the 3.52 reduction requirement and as such is not recommended as an effective PHP, particularly in warmer months where *V. parahaemolyticus* tends to be present in greater abundance.

Re-submersion

Table 62. Log reductions of high quality and high relevance studies: Re-submersion.

Study	PHP Method	Log reduction (log MPN/g)
Jones 2016	Intertidal exposure, Re-submersion	2.73 to 3.21 (total), < 1 (*tdh*+), 2 (*trh*+)
Kinsey 2015	Dry storage, re-submersion	0.2 to 0.3 log reduction or 0.2 to 0.9 log increase

Synthesis of evidence from only studies of high quality and high relevance for re-submersion PHPs are reported in Table 62. Though the above studies have inconsistent results regarding the efficacy of re-submersion, all report that log reductions do not meet the 3.52 threshold. Re-submersion is suggested to be more effective against total *V. parahaemolyticus* than pathogenic strains, after intertidal exposure.

Temperature shock, stress

Table 63. Log reductions of high quality and high relevance studies: Temperature shock, stress.

Study	PHP Method	Log reduction[1] (log CFU/ml)	Survival ratio or rate
Aagesen 2014	Heat shock	Increase < 1	
DePaola 2009	Mild heat	0 to 3	>90% of the mild heat and HHP samples had > 0.04 *Vp* per g, 750-fold lower than specified for nondetectable levels (<30/g)
Zarei 2014	Heat shock		1.1 to 2.4%

Synthesis of evidence from only studies of high quality and high relevance for temperature shock or stress PHPs are reported in Table 63. Mild heat has the potential to decrease *V. parahaemolyticus* growth; however, with such inconsistent findings regarding other heat treatments, more information is needed to confirm whether mild heat can predictably reduce abundance and risk of illness.

DISCUSSION

Overview

Using systematic review methods, 9,587 studies were originally identified using the search strategy; 1,444 studies were identified as potentially relevant; and 232 studies were included for full-text review. Of those studies, 114 were categorized as post-harvest; 26 studies were further excluded as they met reasons for exclusion during full-review. Data was extracted and quality and relevance evaluated for 88 studies. Of the 88 studies, 26 were considered high quality and high relevance according to objective evaluation criteria.

Overall, more research should focus on those PHP methods for which few or no high quality and high relevant studies were available, such as, mild heat, acid, irradiation, relay, depuration, and icing. More research on growth and inactivation rates of pathogenic strains found in Chesapeake Bay and Washington state oyster harvesting waters, under realistic test and environmental conditions, would be informative.

Strengths of high quality studies involve their level of rigor and reliability, and strengths of high relevance studies involve their potential to contribute to regionalization of the existing *V. parahaemolyticus* QMRA and suggest changes to the model parameters and framework structure with updated information. Limitations of low quality studies were most commonly lack of a control group, and the precision of enumeration method used. Given the study design used, a control group was not always feasible, as in observational studies. Regarding precision of enumeration, some studies may have lacked the resources or expertise to use a selective enrichment-MPN method followed by a molecular method, such as PCR.

Limitations of low relevance studies were most commonly region and assumptions testing. As many studies that examined the effectiveness of PHPs were conducted in controlled settings in the laboratory, region was not a disqualifier as it was for the Harvest and Consumption Modules. Illness caused by *V. parahaemolyticus* in raw shellfish affects consumers throughout the world, with the highest incidence occurring in Asia; therefore, it was expected that researchers

across the United States and in other countries would have published studies on a variety of PHPs. The PHP interventions reviewed in this thesis were selected because they were considered to represent PHPs currently in use, or they can reasonably be anticipated to be used in the future, in the Chesapeake Bay and Washington. These findings were considered generalizable to the Chesapeake Bay and Washington state. Still, it was important to identify studies that were conducted in the regions of interest during relevance evaluation.

As the assumptions of the FDA QMRA were formed to account for incomplete data and information gaps at the time, studies may not have directly challenged the assumptions of the FDA QMRA (and/or the WDOH QMRA) if they differed in scope and purpose. For example, HHP and irradiation studies did not tend to address assumptions of the FDA QMRA, because the assumptions were overall not relevant to those studies. The FDA QMRA assumed that growth rates of pathogenic and non-pathogenic *V. parahaemolyticus* strains were similar, but studies on HHP and irradiation did not measure growth rates. Instead, HHP and irradiation studies tended to provide data on log reduction of pathogenic and/or non-pathogenic *V. parahaemolyticus* strains achieved by the intervention, so those studies may support a change to existing parameters.

Using only studies classified as high quality and high relevance would restrict the number and type of studies available and would restrict the variety of data and test conditions available that can improve understanding of the variability and uncertainty of the effective of different PHPs on *V. parahaemolyticus* in raw oysters. Instead, a weighted approached is recommended, with studies of high quality and high relevance weighted the highest, followed by moderate studies and then low quality and low relevance studies, in informing model parameters and inputs.

Comparison of Weight of Evidence to FDA QMRA

The FDA QMRA reported log reductions associated with mitigation strategies for reducing levels of *V. parahaemolyticus* in raw oysters. The body of evidence available on the effectiveness of mitigation strategies has increased since the FDA QMRA, for which the most

recent post-harvest studies included were published in 2002. For the FDA QMRA, one study was used to inform log reduction from irradiation; four studies were used for HHP; two studies were used for hot water pasteurization followed by cold shock; three studies were used for mild heat treatment; two studies were used for freezing; two studies were used for immediate refrigeration; one study was used for relaying; and six studies were used for depuration. In contrast, for this thesis, 9 studies were identified on irradiation; 13 on HHP; 14 on temperature shock or stress; 24 on cold storage (including freezing); seven for relaying; and 12 on depuration. Some trends regarding the effectiveness of high pressure, irradiation and depuration have remained largely consistent, while other trends are not consistent with the current state of evidence (Table 64). Notably, the use of thermal processes such as mild heat, hot water and cold shock were reported to achieve greater than 4.5 log reductions in the FDA QMRA; current findings (Tables 63, 64) generally suggest lower log reductions.

Table 64. Comparison of PHP Effectiveness: 2005 vs. 2018 state of evidence.

Method	**Log reduction** FDA 2005	**# studies**	**Closest equivalent Method** for current SR[2]	**Log reduction** (high quality, rel. for current SR)	**# studies**	**Log reduction** (all for current SR)	**# studies**
Irradiation	6	1	Irradiation	> 7	2	>7	9
Ultra high pressure	6	4	HHP	> 7	6	>7	13
Hot water/cold shock	5	2	Heat shock	Increase	2	6 (increase also observed)	7
Mild heat	≥ 4.5	3	Mild heat	3	1	3	1
Freezing and frozen storage	2	2	Freezing and frozen storage	To < LOD	1	4.5	2
Immediate refrigeration	≤ 1	2	Cold storage	2	7	3	22[1]
Relaying	< 1	1	Relaying	>2	2	4.5 (increase also observed)	7
Depuration	0 to 2	6	Depuration	3	3	5	12

[1]This count excludes the two studies under freezing, frozen storage.

[2]Systematic Review

Table 64 compares reduction effectiveness reported in FDA 2005 with findings from the current review, reporting approximate maximum log reductions achieved, for the closest equivalent methods (applied alone) based on descriptions provided in FDA 2005. Reductions associated with mild heat, heat shock, and relaying from studies determined to be high quality and of high relevance disagree with the log reductions reported in the FDA QMRA (Table 58). As more data is available for this review than was available and considered for the 2005 QMRA, discrepancies were anticipated and can inform recommendations to changes to existing guidelines about PHPs found effective using updated methodology. Post-harvest methods that consistently attain 3.52 log reductions include high pressure, irradiation, and disinfectants. Specific conditions used to meet 3.52 log reductions are reported from high quality, high relevance studies:

- Cold storage: there were no studies that met 3.52 log reductions through cold storage alone. Applying 225 to 300 MPa for 2 minutes after cold storage of 4°C for 2 days, 10°C for one or two days, or -18°C for two weeks, reduced *V. parahaemolyticus* by approximately 5.4 to 6 log MPN/g (Ye 2013).
- HHP: Application of 250 MPa for 2 minutes at 22 to 24°C reduced *V. parahaemolyticus* counts by 5 log MPN/g, and 300 MPa for 2 minutes reduced counts by 6.9 logs (Ye 2013). Treatment of 293 MPa for 120 seconds at 8°C reduced *V. parahaemolyticus* by greater than 3.53 log MPN/g (Ma 2011). Application of 250 MPa for 2 minutes or 300 MPa for 1 minute also reduced populations by more than 3.52 log CFU/g, though log reduction varied across incubation temperatures with pressure resistance tending to increase at higher incubation temperatures (Ye 2011). 275 MPa for 2 minutes at 21°C reduced *V. parahaemolyticus* up to 4.6 log MPN/g, while 300 MPa caused 7 log reductions (Ye 2012). Such findings verify that current industry practices do not decrease *V. parahaemolyticus* to less than the limit of detection. Mild heat following high pressure increased the HHP efficiency in inactivating *V. parahaemolyticus* (Ye 2012).

- Irradiation: Thulipa 2011 found that irradiation at 0.7 kGy achieved approximately 4.3 log CFU/g reductions.

CONCLUSIONS

Systematic review methods were used to select and evaluate studies for the post-harvest module of the exposure assessment to inform a future QMRA of *V. parahaemolyticus* in raw oysters. Of 88 studies selected for inclusion in the post-harvest module, 12 were both high quality and high relevance according to objective evaluation criteria.

At this time, it cannot be recommended that only studies of high quality and high relevance be used to inform the post-harvest model for a future QMRA. Doing so would severely restrict the amount of valuable information that can used to address information gaps and uncertainties from the existing FDA QMRA. Instead, it is recommended that studies of high quality should be weighted more heavily than studies of moderate quality (fully meet at least 3 key quality criteria and at least fully meet one and partially meet one relevance criteria), which in turn should be weighted greater than low quality studies (fully meet two or less key quality criteria, fully meet less than one relevance criteria), in informing parameters and inputs of the post-harvest model. This promotes the use of available information weighted according to quality and relevance that can be used to recommend regulatory changes to help stem the tide of increasing illnesses.

Qualitative and quantitative comparison of study findings for various PHPs inform potential new inputs that should be included in a future risk assessment model. Suggested changes to the Exposure Assessment schematic find that PHPs should be included with inputs for inactivation rate and log reduction (Figure 4).

Inclusion of parameters and uncertainty distributions around the inputs for log reduction, growth rate during cooldown, and growth rate during storage, would enhance model ability to predict pathogenic *V. parahaemolyticus* at retail and consumption. Implementation of these

changes would enable consideration of more opportunities for growth as well as reduction during critical control points of *V. parahaemolyticus* post-harvest, promoting a more comprehensive exposure assessment that reflects the current state of the evidence. For instance, growth rates for total and pathogenic *V. parahaemolyticus* extracted from selected studies are reported in Table 65. These data challenge the proposed *V. parahaemolyticus* growth rate of 0.84 $\log_{10}$ / hr in broth at 26°C used in the FDA QMRA.

Table 65. Total and pathogenic V. parahaemolyticus growth rates.

Study	**Growth Rate – Total**[1]	**Growth Rate – Pathogenic**[1]	**Units**	**PHP**
Mudoh 2010	0.08 to 0.17 (at 10 and 15°C)	-.0007 to -.0019 (at 5°C)	Log CFU/hr	Cold storage
Kim 2012		-0.005 to 1.534 (strain ATCC 33844)	Log CFU/hr	Temperature (13 to 36°C)
Fernandez-Piquer 2010	0.036 to 0.205		Log MPN/hr	Storage (23 to 28°C)
Fernandez-Piquer 2011	-.006 (3.6°C); .282 (30.4°C)		Log CFU/hr	Cold Storage; storage
Parveen 2013	-0.0036 to 0.022		Log CFU/hr	Cold storage (5 to 15°C)
Wang 2018b		2.15 (ATCC 17802); 1.51 (ATCC 33847)	Log CFU/hr	Oyster food martrix (Gompertz model)

[1]For studies that reported mean $\pm$ standard deviation (SD), the range indicates the minimum mean – SD reported, and the maximum mean + SD reported.

Future Directions

Data Extraction

Information that can be extracted in future work to inform the updated and regionalized Dose-Response, Harvest, and Consumption modules is listed in Tables A21 – A23 in the Appendix.

Study Quality, Relevance Evaluation

Evaluation of the other modules was beyond the scope of this thesis. However, study evaluation criteria formed for the post-harvest module could be used and adjusted to reflect the specifications of the other modules. Anticipated evaluation criteria are as follows:

Dose-Response Assessment: The dose-response model characterizes the relationship between the amount of *V. parahaemolyticus* consumed and the frequency and severity of illness. Studies will be evaluated on the extent to which they characterize variability (defined as differences of an attribute among members of a population) and uncertainty (lack of knowledge on a fixed quantity, abundance of *V. parahaemolyticus*, expressed as a probability distribution) in the dose-response relationship (FDA 2005). Studies will be evaluated on their consideration of different virulence factors, such as Type III Secretion Systems (T3SSs) and use of virulence detection methods to determine the dose-response relationship. Further, rates of under-reporting and under-diagnosed illnesses will be used to update and inform the magnitude of the multiplier used in the FDA QMRA.

Additionally, how the study characterizes extrapolation from feeding study doses to typical doses that would be ingested as part of an overall food matrix will also be considered. Though the FDA QMRA selected the Beta-Poisson model because it alone met the FAO/WHO mechanistic criteria of no threshold for bacterial microorganisms, new studies may better characterize the relationship with other models. Qualitative evaluation of the models will consider how they address information gaps from the FDA QMRA, and how they consider susceptible

populations in deriving the dose-response relationship. Finally, animal feeding studies will be evaluated based on the extent to which measurements of the severity of illness in animal studies correspond with definitions of human illness on which reporting statistics are based. Some studies identified in chapter 2 indicate the potential usefulness of infant rabbit (Ritchie et al. 2012) and zebrafish (Paranjype et al. 2013) models to explain pathogenesis of *V. parahaemolyticus*.

Harvest: It is anticipated that the Harvest model will be specific to Chesapeake Bay and Washington state, based on the different regional methods of oyster harvest, presence of virulence markers, and environmental determinants of growth and abundance. Studies from Washington state will be additionally evaluated on their coverage of different types of intertidal harvest methods (how many different methods do they consider, and are those methods practiced widely?) and consideration of variability in growth of *V. parahaemolyticus* between and within those methods.

The FDA QMRA harvest module included studies that contained multistate, long-term historical data from all seasons. Most oyster samples with detectable *V. parahaemolyticus* levels occur from April to October (Parveen et al. 2008), and *V. parahaemolyticus* largely remains in the sediment and not the water column in winter months (Su and Liu 2007). As such, it may be unnecessary to require year-round monitoring. During study quality evaluation, studies will be evaluated on the collection of monitoring data from April to October, rather than year-round.

Consumption Behaviors: Studies will be evaluated on the region and number of oyster consumers included in the study and whether they report the harvest location of oysters. Like the other models comprising the exposure assessment component, studies that address assumptions for the Consumption model will also be evaluated based on quality of data and analysis. Inclusion of consumer health status will be also be evaluated (as a binary yes or no characteristic) and the information used to inform the synthesis of evidence.

Synthesis of Evidence

The state of the evidence may suggest added value in changing the modules for dose-response, harvest, and consumption based on recent studies that address uncertainties or information gaps limiting previous risk assessments, resulting in changes to the risk assessment framework for a future QMRA.

Dose-Response: Both the FDA QMRA and WDOH 2014 used the Beta-Poisson dose-response model. Using the studies identified in Chapter 2, Synthesis of evidence from human and animal feeding studies that are anchored with epidemiologic data may suggest selection of a different model for the dose-response relationship, such as the Gompertz or Probit model, or a modified relationship using the Beta-Poisson distribution. Consideration of new rates of under-diagnosed or unreported illnesses from *V. parahaemolyticus* may substantially alter previous risk predictions.

Bacteria within the food matrix are anticipated to follow a Poisson distribution, while the probability of infection follows a Beta distribution, leading to a Beta-Poisson dose-response model (FDA 2005). The FDA QMRA used a likelihood ratio goodness-of-fit test to assess how well the model fit the data; all models explored, the Beta-Poisson, Gompertz, and Probit, provided adequate statistical fit. Differences between the models were greater at low doses. The maximum likelihood estimate for the ID50 was 2.8×10^6 for the Beta-Poisson model, 4.0×10^6 for the Gompertz model, and 3.2×10^6 for the Probit dose-response model. All three models had comparable uncertainty (residual) distributions, so the Beta-Poisson model was selected as it alone met the FAO/WHO mechanistic criteria of no threshold (FDA 2005). With no threshold confirmed to exist for bacterial microorganisms at the time of the FDA QMRA, this choice was deemed most appropriate. New research may suggest that a different approach is more fitting.

Uncertainty of the Beta-Poisson model approximation is greater at low doses, meaning the difference between the Beta-Poisson distribution formula and the actual function is greater at low doses of *V. parahaemolyticus* and may overestimate risks (Teunis and Havelaar 2000). This

discrepancy presents concerns: if the model used in previous risk assessments does not provide a good fit at low doses, doses that are more commonly experienced by consumers, the model's ability to forecast risk of illness is limited. Future work should reinvestigate model choice to understand if other models previously considered (Gompertz, Probit) can improve forecasting capabilities and provide a better fit at lower, more relevant doses.

Severity of disease is strongly positively associated with the presence of underlying medical conditions found in susceptible populations, such as alcoholism and liver disease (Daniels et al. 2000) or immunosuppression. Other medical conditions shown to associate with fatal gastroenteritis or septicemia were renal disease, vascular disease and diabetes (Daniels et al. 2000). General health status, nutritional status, or physical stress also affects immune response. Immune status (and the intrinsic factors it is influenced by) affects occurrence and severity of disease. Human clinical feeding trial data were used to develop the dose-response model in the FDA QMRA, but the studies did not collect information on immune status of subjects. Case studies identified in Chapter 2 should be used to characterize the dose-response relationship for vulnerable and sensitive subpopulations.

The existing FDA QMRA excluded animal data from its dose-response model in part because severity of illness measures in animals did not align with severity of illness measures in humans in relevant studies the authors reviewed. The publication of recent animal studies using the oral route of exposure to examine intestinal pathology and diarrhea caused by *V. parahaemolyticus* (Ritchie et al. 2012) may have the potential to inform human response to *V. parahaemolyticus.*

While several animal studies observed that TDH-negative strains have virulence potential, how virulence potential of applies to humans remains unknown. However, there is potential utility in using animal studies to extrapolate to human dose-response estimates, and future work will extract data from and evaluate the animal model studies identified in Chapter 2.

Use of epidemiological and surveillance data identified in Aim 1 will further inform the dose-response relationship and epidemiological adjustment to the model.

Harvest: The state of the evidence may suggest that changes to the Harvest Module are necessary according to the identified studies. Decisions on parameter selection should be reevaluated based on the recent body of evidence from the identified studies. For instance, recent research finds that optimal growth temperatures range from 30 to 40°C, with greater abundance in warm seasons (Vezzulli et al. 2013). When water temperatures are under 15°C, levels of *V. parahaemolyticus* are insufficient to cause outbreaks (FAO/WHO 2011). As such, consideration of studies that did not sample during colder months may not represent a sufficient reason for exclusion. Recent studies are anticipated to report more data on parameters that may influence the prevalence and persistence of pathogenic *V. parahaemolyticus* strains, such as water temperature, salinity, total suspended solids, turbidity, dissolved oxygen, tidal flushing, zooplankton, as well as shellfish species and physiology, and genetics of the microorganism. Pulsed-field gel electrophoresis (PFGE) analysis is increasingly used to examine serotypes and virulence factors from isolates in Washington state, Chesapeake Bay, and around the world to inform relatedness of strains with previous outbreaks and environmental persistence (Chiu et al. 2007). Quantitative synthesis using statistical methods such as Bayesian analysis may be considered for evidence integration.

Assumptions made for the Harvest model of the original QMRA should be updated to reflect recent literature. Pathogenesis can be characterized by multiple virulence factors (Zhang and Orth 2013); and percent pathogenicity may not be constant throughout the year, as warmer water temperatures may be linked to more virulent populations (Martinez-Urtaza et al. 2010). Future work would consider virulence factors promoting the rise of pathogenic serotypes involving the *tdh* and *trh* genes and other virulence factors not originally considered (Letchumanan et al. 2014).

QMRA modelling could also consider the impact that warming sea surface temperatures due to climate change would have on risk of illness, as well as other weather events, such as El Niño and hurricanes. The 1997 *V. parahaemolyticus* epidemic that spread from Peru to Chile and caused 10,000 cases of gastroenteritis from shellfish consumption coincided with a major El Niño episode (Martinez-Urtaza et al. 2016). El Niño water movements correlated with *Vibrio* infections and warmer sea surface temperatures (Martinez-Urtaza et al. 2016). Storm events such as hurricanes are hypothesized to distribute benthic *V. parahaemolyticus* populations into the water column through flushing from high precipitation and sediment resuspension from high winds and wave energy (Shaw et al. 2014). Other areas in the U.S. have observed increases in storm-related *Vibriosis* cases. Shaw et al. (2014) sampled surface water, sediment, and Eastern oysters from the surface and near-bottom at aquaculture sites in the Chesapeake Bay before and after Hurricane Irene in August 2011. Hurricane Irene was a significant wind event and caused sediment resuspension and heavy precipitation (Shaw et al. 2014). Though *V. parahaemolyticus* increased substantially following the hurricane, there were no statistically significant changes in *V. parahaemolyticus* abundance by location or date for all samples collected, pre- to post-hurricane (Shaw et al. 2014). Though *V. parahaemolyticus* in oyster tissues did not correlate with environmental measurements, sediment and surface water *V. parahaemolyticus* did correlate with environmental measurements, such as secchi depth and tidal height (Shaw et al. 2014). Surface water *Vibrio* concentrations increased 7 to 11 times at four days after the hurricane, while *Vibrio* concentrations decreased 5 to 10 times on day 4 post-storm (Shaw et al. 2014). As oysters reduce or stop filtration during conditions of high suspended solids and resume filtration activities when suspended solids return to normal levels, that may have contributed to the decrease in oyster *V. parahaemolyticus* after four days (Shaw et al. 2014). As climate change is expected to increase the frequency and severity of storm events, this poses important implications for future *Vibriosis* outbreaks.

Consumption Behaviors: In future work it would be insightful to compare studies of raw oyster consumption patterns in states connected to the Washington state and Chesapeake Bay oyster-harvesting areas with the data on raw oyster consumption patterns from Florida used in the FDA QMRA, considered nationally representative. The preliminary literature review has identified a study that documents raw oyster consumption behaviors and trends in Washington State from 2014-2015 (Cheney 2016). This study was aimed at informing the Washington State Department of Health vibriosis risk assessment models, and reports data from a raw oyster consumption survey distributed to the Seattle area. The survey was distributed to participants at Taylor shellfish restaurants through survey cards, as well as online. Though the consumer preference data is regionalized, the study found that consumer preferences reported in the survey differed from sales data. Use of retailer sales data may provide heightened accuracy on consumption behaviors of oysters harvested in Washington state and Chesapeake Bay.

In Survey Question 2, respondents indicated that on average they tend to eat 7.32 raw oysters per sitting (Cheney 2016). Survey card participants ate on average 6.81 raw oysters while online participants had an average serving size of 7.88 raw oysters due to more outlying data points. Survey data differed from sales data (Taylor Shellfish Farms' Seattle restaurants reported a serving size between 3.8-4.5 raw oysters). Additionally, online participants may have the tendency to exaggerate average serving size, as there were more statistical outliers among online respondents. Cheney attributed this as potential "social desirability bias," they remembered occasions where they ate a greater number of oysters per sitting than usual, instead of a more representative number across multiple meals. In-restaurant participants were likely influenced by their most recent order (Cheney 2016).

In Survey Question 4, respondents indicated they prefer to eat oysters harvested in the winter (71.4%), fall (68.8%) and spring (67.7%) (Cheney 2016). Only 44.3% participants said they typically eat raw oysters in the summer. However, key informant testimony indicated that raw oyster sales peak during summer months (WDOH 2015); this corresponds to seasonal sales

information. Illnesses also tend to occur most frequently from oysters harvested during summer months (Cheney 2016).

In Survey Question 7 regarding size of raw oysters consumed, 14.4% of respondents indicated they prefer to eat petite oysters (< 2.5 in), 34.4% preferred extra small (2.5-3 in), 26.8% preferred small (3-4 in), 3.8% preferred medium or large (< 4 in), and 20.6% had no preference (Cheney 2016). These responses correspond to WA State data on oysters produced for raw consumption but may not be applicable to other harvesting regions. Overall, Cheney (2016) provides insight into consumption preferences in Washington state that differ from consumption behavior patterns applied in the FDA 2005 QMRA.

For the FDA QMRA, the 1994 Florida survey data was assumed to be representative of trends nationally. The most common responses for serving size were 6, 12, and 24 oysters; 12 oysters were the most frequent serving size reported, with variation between 6 and 12 oysters considered typical. In contrast, average serving size reported in Cheney (2016) was approximately 7 oysters. Additional studies and/or market surveys may further modify or inform assumptions used in the Consumption model.

Final Remarks

In conclusion, based on recent literature identified and evaluated using systematic review, HHP and irradiation emerge as effective PHPs that can consistently achieve greater than 4 log reductions in *V. parahaemolyticus* abundance. Findings of this thesis indicate that future changes to the post-harvest model of the exposure assessment should include parameters for growth rate during storage; inactivation rate during cooldown; and log reduction achieved through PHP applied (Figure 4). As this thesis used systematic review methods, it is anticipated that the methods adapted and developed here may be used to continue to update existing models and support future QMRAs of *V. parahaemolyticus* in raw oysters. As literature continues to emerge on the pathogenicity of *V. parahaemolyticus* and its environmental determinants, implications of risk to more vulnerable and susceptible subpopulations, raw oyster consumption patterns, and the

effectiveness of hitherto less-studied PHPs, the methods described here provide a roadmap for systematic incorporation of new data to better inform risk management strategies.

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APPENDIX

Search strategy for harvest and post-harvest modules

PubMed

Table A1. Search strategy for harvest and post-harvest modules; PubMed.

1.	"Vibrio parahaemolyticus"[Mesh] OR "Vibrio parahaemolyticus"[tw] OR "v. parahaemolyticus"[tw] OR "Vibrio parahemolyticus"[tw] OR "V. parahemolyticus"	
2.	"Vibrio Infections"[Mesh:NoExp] OR Vibrio Illness*[tw] OR Vibriosis[tw] OR Vibrioses[tw] OR Vibrio Infection*[tw]	
3.	Vibrio*[tw] AND harvest*[tw]	
4.	#1 OR #2 OR #3	3,748 results

Searched and all results downloaded on 7/26/2018.

Embase

Table A2. Search strategy for harvest and post-harvest modules; Embase.

1.	('vibrio parahaemolyticus'/exp OR 'vibrio parahaemolyticus' OR 'vibrio parahaemolyticus infection'/exp OR 'vibrio parahaemolyticus infection' OR 'vibrio parahaemolyticus':ti,ab,kw OR 'v. parahaemolyticus':ti,ab,kw OR 'vibrio parahemolyticus':ti,ab,kw OR 'v. parahemolyticus':ti,ab,kw) AND [2004-2018]/py	
2.	('vibrio parahaemolyticus infect*' OR 'vibriosis'/de OR 'vibrioses') AND [2004-2018]/py	
3.	vibrio*:ab,ti,kw AND harvest*:ab,ti,kw AND [2004-2018]/py	
4.	((('vibrio*' OR 'vibrio parahaemolyticus' OR 'v. paraehmolyticus') NEAR/2 (illness* OR infect*)):ab,ti,kw) AND [2004-2018]/py	
5.	#1 OR #2 OR #3 OR #4	4,196 results

Searched and all results downloaded on 8/22/2018.

Scopus

1. "Vibrio parahaemolyticus" OR "v. parahaemolyticus" OR "Vibrio parahemolyticus" OR "V. parahemolyticus" OR "Vibrio Infect*" OR vibrio AND illness* OR vibriosis OR vibrioses OR (vibrio* AND harvest*) AND (LIMIT-TO (PUBYEAR , 2018) OR LIMIT-TO (PUBYEAR , 2017) OR LIMIT-TO (PUBYEAR , 2016) OR LIMIT-TO (PUBYEAR , 2015) OR LIMIT-TO (PUBYEAR , 2014) OR LIMIT-TO (PUBYEAR , 2013) OR LIMIT-TO (PUBYEAR , 2012) OR LIMIT-TO (PUBYEAR , 2011) OR LIMIT-TO (PUBYEAR , 2010) OR LIMIT-TO (PUBYEAR , 2009) OR LIMIT-TO (PUBYEAR , 2008) OR LIMIT-TO (PUBYEAR , 2007) OR LIMIT-

TO (PUBYEAR , 2006) OR LIMIT-TO (PUBYEAR , 2005) OR LIMIT-TO (PUBYEAR , 2004))

Searched and sorted by relevance on 8/22/2018; first 2,000 results (of total 10,613) downloaded, as site only permitted download of first 2,000 documents.

Searched and sorted by date (newest) on 8/24/2018; first 2,000 results (of total 10, 627) downloaded.

NAL Agricola

1. "Vibrio parahaemolyticus" OR "v. parahaemolyticus" OR "Vibrio parahemolyticus" OR "V. parahemolyticus" OR (Vibrio* AND harvest)

   From 2004, Advanced search, Keyword Anywhere for all terms.

Search conducted and one total result downloaded on 7/28/2018.

Science.gov

Table A3. Search strategy for harvest and post-harvest modules; Science.gov.

1.	"Vibrio parahaemolyticus" OR "v. parahaemolyticus" OR "V. parahaemolyticus"	349 results
2.	"Vibrio Infect*" OR Vibrio Illness* OR Vibriosis OR Vibrioses	340 results
3.	Vibrio* AND harvest*	492 results

Search conducted and all results, 1,181 total, downloaded on 7/28/2018.

Google Scholar

Table A4. Search strategy for harvest and post-harvest modules; Google Scholar.

1.	"Vibrio parahaemolyticus" OR "v. parahaemolyticus" OR "V. parahaemolyticus"	16,500 results searched 7/28/18
2.	"Vibrio Infect*" OR Vibrio Illness* OR Vibriosis OR Vibrioses,	16,900 results searched 7/29/18
3.	Vibrio* AND harvest*	14,700 results searched 7/29/18

Search conducted and first 200 results for each search downloaded.

Search strategy for consumption module

Google Scholar

1. Raw oyster consumption behavior*

Search conducted and first 200 results (of total 15,700 results) downloaded on 7/31/2018.

PubMed

Table A5. Search strategy for consumption module; PubMed.

1.	"Food Quality"[Mesh] OR "Feeding Behavior"[Mesh] OR "Diet Surveys"[Mesh] OR food*[tw] OR diet[tw] OR diets[tw] OR survey*[tw] OR questionnaire*[tw] OR consume*[tw] OR consumption*[tw]	
2.	oyster*[tw] OR raw shellfish[tw]	
3.	#1 AND #2	1,295 results

Search conducted and all results downloaded on 7/26/2018.

Embase

Table A6. Search strategy for consumption module; Embase.

1.	('oyster'/exp OR 'raw shellfish') AND [2004-2018]/py	
2.	('food quality'/exp OR 'feeding behavior'/exp OR 'diet surveys'/exp OR 'food*' OR 'diet'/de OR 'diets'/de OR 'survey*' OR 'questionnaire*'OR 'consume*' OR 'consumption*') AND [2004-2018]/py	
3.	#2 AND #3	1,334 results

Search conducted and all results downloaded on 8/22/2018.

Table A7. Reasons for exclusion from Post-Harvest Module during full-text review.

	Reason for Exclusion	Studies
Accessibility	3	Chiang 2012; Flores-Primo 2015; Maruyama 2005
Bacteria genus/ species mismatch	3	Cao 2009; Costa 2014; Lingham 2016
Duplicate	6	Chae 2007 (thesis for Chae 2009); Elmahdi 2017; Huang 2012; Kim 2012; Phuvasate 2014; Yang 2009 (duplicate for Su 2010)
Methods	3	Fernandez-Piquer 2013; Ghazaleh 2014; Lacey 2015
Non-handling intervention	4	Fang 2015; Rong 2014; Wenneberg 2010; Xi 2014
Review	4	Baker 2016; Ronholm 2016; Su 2013; Teplitski 2009;
Vibrio mechanistic study	2	Urano 2006; Hamamoto 2010
Wrong animal species	1	Barile 2009
Total Excluded	26	

Table A8. Qualitative Findings: Acid.

Study	PHP Method	Qualitative Findings
Chiang 2008a	Ethanol shock, organic acids	Increased susceptibility to organic acid with increasing ethanol shock duration.
Chiang 2014	Acid adaptation, Heat stress	Survival of acid-adapted strains to heat stress was sig. greater than of non-adapted strains; acid adaptation increased thermotolerance of *Vp*. Survival between acid-adapted and non-adapted strains of *Vp* to cold stress did not differ sig.
Chiang 2014	Acid adaptation, High salinity	Strain differences in survival between non-adapted and acid-adapted cells to high salinity.
Hasegawa 2013	Acid stress	Tolerance against acid stress in *tdh*- and *trh*- strains sig. lower than in *tdh*+ and/or *trh*+ strains.
Kalburge 2014	Acid pre-adaptation, acid stress	Mild acid preadaptation phase sig. increased survival to lethal acid stress. Differences in increased survivability to acid stress across strains.
Lin 2004	Lactic acid	In general, cold shock treatments reduced acid resistance of *Vp* in subsequent exposure to lactic acid. No sig. difference in survival between control and cold-shocked cells, but cold-shocked cells died off more quickly than control cells with lactic acid exposure. Cold shock made *Vp* cells more susceptible to acetic acid than lactic acid. Survival of cold-shocked cells of *Vp* to acid stress depends on cold shock conditions and kind of acids present.
Lin 2004	Acid, cold shock	Cold shock at 15°C decreased *Vp* acid tolerance more than cold shock at 20°C.
Whitaker 2010	Acid	*Vp* is sig. more toxic when grown at 1% than 3% NaCl.
Wong 2004	Acid	No sig. decrease in survival ratio in starvation-adapted or starvation-low salinity adapted cells.
Yeung 2004	Acid stress	Relative survival of clinical and food isolates not sig. different. Survival of acid-adapted or non-adapted cells to acid challenge varied based on cell phase. No *Vp* strains able to reproduce at pH 4.2.

Table A9. Qualitative Findings: Cold Storage. Part 1.

Study	PHP Method	Qualitative Findings
Burnham 2009	Cold storage	Strain-to-strain differences in growth and survival for *Vp*, with differences sig. in some cases. *Vp* survived but did not grow when stored at 5°C, but grew at 8 and 10°C.
Chang 2004	Heat shock, Cold storage	Survival rate differed when stored at 5 or -18°C.
Chiang 2006	Ethanol shock, Cold storage	Survival after storage did not differ sig, between cells that were ethanol-shocked and controls.
Chiang 2006	Ethanol shock, frozen storage	Ethanol shock made cells more susceptible to cold storage.
DePaola 2009	Freezing, frozen storage	LR achieved to reach non-detectable levels. At retail, all samples < LOD. Differences in *Vp* levels between processor and retail for freezing were stat. sig. At processor, some samples > 30 MPN collected.
Drake 2008	Cold storage	Data on growth rates suggest that pathogenic *Vp* multiplies more rapidly at lower temperatures (10 to 25°C) than non-pathogenic *Vp*. No stat. sig. differences in D-values and recovery of *Vp* strains across different culture media or conditions, regardless of virulence factors. All *Vp* strains detectable for 25 days.
Drake 2008	Cold storage; Starvation, cold stress	Non-pathogenic strains had sig. greater survivability and recovery than pathogenic strains when subjected to starvation.
Fernandez-Piquer 2010	Cold storage	Pacific oysters stored at 15,18 and 24°C didn't observe sig. increase in *Vp* levels.
Fernandez-Piquer 2011	Cold storage	Oysters stored at $\geq$ 18.4°C had *Vp* growth. Growth rate increased with increasing temp.
Huang 2018	Cold storage	Study estimated overall incidence rate of *Vp* infection could be reduced by 67% if processing temps < 12°C.
Jones 2017	Cold storage, icing	Mean *Vp* levels in oysters were lowest in samples immediately iced, and highest in 5 hr ambient stored then refrigerated samples.
Liao 2017	Cold storage	*Vp* strain with *trh* gene had poor survival during cold storage.
Lin 2004	Cold storage	*Vp* survival decreased as storage time increased but decrease greater in control than cold-shocked cells.
Lin 2004	Cold shock, cold storage	Regardless of cold shock treatment, *Vp* declined more at -18°C than 5°C storage.
Liu 2009	Freezing, frozen storage	Flash freezing had little effect on inactivating bacteria (<1 log reduction). Subsequent cold storage reduced *Vp.*
Mudoh 2010	Cold storage	Growth rate of pathogenic *Vp* substantially greater than total *Vp* growth rate. Total and pathogenic *Vp* multiplied more rapidly in Eastern oysters than Asian oysters (*Crassostrea ariakensis*).
Mudoh 2014	Cold storage	No stat sig difference in growth of total *Vp* at 5 and 10°C. No increase in growth at either temp.

Table A10. Qualitative Findings: Cold Storage. Part 2.

Study	PHP Method	Qualitative Findings
Parveen 2013	Cold storage, HHP	Total *Vp* below LOD (10 CFU/g) at 5 and 10°C after 504 h storage.
Prapaiwong 2009	Cold storage	Some *Vp* found during cold storage following HHP or quick freezing across seasons.
Shen 2009	Cold storage	Log reductions achieved at 5 and 10°C storage but growth at 15°C.
Songsaeng 2010	Freezing, frozen storage	Neither individual quick frozen nor contact plate oysters caused significant changes in *Vp. Vp* abundance was less than 3 MPN/g during storage, indicating the effectiveness of time-temperature treatments controlling the rate of freezing and frozen storage in oysters.
Vasudevan 2006	Cold storage	Wild and mutant strain populations decreased during cold storage but mutant strain was sig. lower than wild strain. Sig. interaction between strain and storage temp on survival.
Wang 2018a	Cold storage	Nonpathogenic *Vp* shows similar growth/ survival behavior to pathogenic strains at same temps. When stored in different media at 5°C, fewer strains survive after 200 h in cold storage.
Wu 2007	Cold storage	After 14 d storage, oysters that were gradually cooled to 5°C over 9.5 h had higher *Vp* counts than oysters placed directly in 5°C.
Wu 2007	Ice water storage	Differences in cold response between clinical and environmental *Vp* strains.
Ye 2013	Cold storage, HHP	*Vp* populations slightly lower in whole-shell oysters than in oyster meat but differences not stat sig. Validation study: HHP treatments at 250 MPa followed by 10-day ice storage; 300 MPa followed by 5-day ice storage; at both pressure levels followed by 7-day frozen storage completely eliminated *Vp* in whole-shell oysters.
Zarei 2014	Cold storage, Chlorine stress	Survival rates of Cl-stressed cells of pathogenic and non-pathogenic strains sig. decreased compared to control cells as storage time at 4°C increased.

Table A11. Qualitative Findings: Depuration.

Study	PHP Method	Qualitative Findings
Aagesen 2014	Depuration, heat shock	Seasonal changes affect oyster ability to clear *Vp*; heat shocked oysters retain higher levels of *Vp* than non-HS oysters. More effective to use depuration on oysters not heat-shocked. Extending depuration time may improve LR.
Chae 2009	Depuration	Decreasing water temp. can increase LR of *Vp* in oyster through depuration, but effect limited to 15°C; 10°C had no advantage over 22°C, and depuration at 5°C had negligible *Vp* reduction.
Larsen 2015	Depuration, high salinity	No sig difference in Vp concentrations between temps used, low temperatures (20°C) tended to be less effective than higher temperatures (22.5, 25°C).
Ming 2018	Depuration; depuration with algae feeding	Depuration sig. reduced *Vp* over 6 days. No sig. difference in *Vp* reduction based on feeding status.
Phuvasate 2012	Depuration with UV and cold temp.	Depuration at 7–15°C for 5 days reduced *Vp* populations in oysters by >3.0 log MPN/g. Depuration of at 2 or 3 °C did not significantly reduce Vp, though slightly greater reductions were observed at 3 than at 2C. Oyster biological activity minimized at temps < 5°C. Storing oysters in ice without any other PHP is not an effective means to inactivate *Vp*.
Phuvasate 2013	Depuration	Salinity $\geq$ 20 ppt is favorable for oyster physiology and filter feeding activity. Greater *Vp* reductions observed in oysters depurated in seawater between 20 and 30 ppt, than in salinity of 10 ppt after 5 days.
Phuvasate 2013	Depuration	*Vp* LRs did vary significantly between type and size of oysters.
Ramos 2012	Depuration, UV light, Cl	*Vp* not detected in recirculated seawater after 48 h depuration with UV light or UV light and Cl. *Vp* was detected in recirculated water of control (just depuration) treatment after 48 hr.
Sobrinho 2014	Depuration with ozone, UV	Depuration did not effectively reduce *Vp* post-harvest.
Su 2010	Depuration	Depuration with refrigerated ASW at 5°C took 96 hrs for 3-log reductions in *Vp* in winter oysters; 144 hrs for summer oyster.
Wang 2010b	Depuration	*Vp* was highest in digestive glands, followed by gills.
Yu 2010	Depuration and recirculation or flow-through, creek relay	*Vp* was sig. reduced in Depuration-recirculation, Depuration-flow through, and creek relay treatments in August, but only creek relay has sig reductions in Sept – Nov. Depuration-recirculation was least effective.

Table A12. Qualitative Findings: Disinfectant. Part 1.

Study	PHP Method	Qualitative Findings
Chang 2004	Ethanol, heat shock	Survival of heat-shocked and control cells decreased with ethanol exposure, survival generally higher for heat-shocked cells. As time of exposure to heat shock increased, survival of ethanol-exposed cells increased.
Chang 2004	Hydrogen peroxide, heat shock	Heat shock significantly increased *Vp* resistance to H2O2. Increasing duration of heat shocking generally increased *Vp* resistance to H2O2 damage. Not consistent with other study findings but used much higher [H202].
Chiang 2006	Ethanol shock	Presence of ethanol resulted in partial inhibition or bactericidal effect on *Vp*, depending on ethanol concentration. 5% ethanol selected as the sub-lethal dosage level for ethanol shock treatment.
Chiang 2006	Ethanol shock, heat shock	Ethanol shock increased thermal tolerance of *Vp* at 47°C. Ethanol-shocked cells had higher survival percentage than control cells. Duration of ethanol shock did not cause difference in survival of ethanol-shocked cells at 47°C.
Chiang 2008a	Ethanol shock	Survival of control and ethanol-shocked cells decreased as exposure time increased. Ethanol-shocked cells had lower survival than controls.
Chiang 2008a	Ethanol shock, organic acids	Ethanol shock changed *Vp* susceptibility to type of organic acid tested compared to controls. Increased susceptibility to organic acid after ethanol shock.
Chiang 2008a	Ethanol shock, Hydrogen peroxide	Ethanol shock increased *Vp* resistance to H202, effect increased as ethanol shock duration increased.
Chiang 2008a	Ethanol shock, NaCl	Ethanol shock increased *Vp* susceptibility to high NaCl.
Chiang 2008b	Ethanol shock	Ethanol shock increased TDH two-fold in cell supernatant. Ethanol-shocked and control *Vp* had same GR in TSB-3% NaCl.
Chiang 2014	Ethanol, acid adaptation	Acid adaptation increased *Vp* resistance to ethanol in strains 690 and BCRC 13025 but not BCRC 13023.
Chiu 2006	Electrolyzed oxidizing water on surfaces	Treatment of EO water for 30–60 s totally inactivated *Vp* on stainless steel, glazed ceramic tile, and plastic cutting board; was less effective on bamboo or wood cutting boards.
Lai 2013	Hydrogen peroxide, cold stress, low salinity	*Vp* had sig. increased resistance to H202 in starved culture; *Vp* had sig. higher susceptibility to H202 after low salinity stress or low salinity and starvation stress, compared to exponential-phase control.
Lin 2004	Hydrogen peroxide, cold shock	After cold shock, *Vp* became more susceptible to H2O2.
Lin 2013	Chlorine disinfectant, heat shock	Heat shock and cold shock increased *Vp* resistance to disinfectants. Greater population reductions at 40°C than at 25°C.

Table A13. Qualitative Findings: Disinfectant. Part 2.

Study	PHP Method	Qualitative Findings
Park 2018	Sodium hypochlorite, Gamma irradiation	60 ppm NaClO + 2.0 kGy effectively reduces 7-log *Vp* without any deteriorative changes of sensory qualities. 60-80ppm NaClO with 0.5-0.9 kGy acheives 5 log *Vp* reduction in oysters.
Takahashi 2016	Sodium hypochlorite	Attached cells more resistant to NaOCl solutions than unattached cells. Rate of inactivation for unattached and attached cells increased with decreasing pH.
Shi 2017	Saline solution	Average recovery rates were higher for polypropylene than for stainless steel. Survival curves under simulated real conditions also found that *Vp* may survive better on polypropylene than stainless steel. Average recovery rates from wet surfaces was more variable than from dry surfaces.
Wang 2010a	Chlorine dioxide	After 4 h incubation with ClO2, *Vp* only detected in digestive glands. All *Vp* in oysters disinfected within 6 hr. *Vp* levels were highest in digestive glands compared to all tested tissues (gills, individual oyster). Disinfection rate affected by water temperature, high-protein food might interfere with antimicrobial activity. Sig. difference in bacterial populations between gills and ASW, and digestive glands and individual oysters. For cold storage following ClO2 treatment, shelf life of oysters extended to > 12 d after 6 h of CIO2 treatment at 4°C. No *Vp* contamination detected in individual oysters.
Zarei 2014	Chlorine stress, sodium chloride	Cl-stressed cells of pathogenic and non-pathogenic strains had sig. lower survival rates compared to control cells. Comparing survival rates of Cl-stressed cells of both strains showed no significant difference, similar to normal strains.

Table A14. Qualitative Findings: High Pressure.

Study	PHP Method	Qualitative Findings
Chen 2006	HHP	Treatment time did not sig affect Dp values but affected LR (doubling treatment time approx. doubled LR). Critical pressure level at which inactivation occurred (≥ 0.5 log reduction) was 200 MPa for *Vp* for 10 mins at 21.5°C.
Chen 2007	HHP	*Vp* most sensitive to pressure of the pathogens studied.
DePaola 2009	HHP	> 70 % of HHP samples reduced to < .04/g.
Hu 2005	HHP	Weibull frequency distributions found to predict HHP inactivation more accurately in pure cultures and inoculated oyster samples.
Koo 2006	HHP	High pressure effective for reducing *Vp*. O3:K6 is most pressure resistant of serotypes tested.
Kural 2008	HHP, varying temperature	Effect of cold temperature on LR achieved varied with pressure.
Ma 2011	HHP	HPP of 293 MPa for 120 s at 8+1 C could achieve > 3.52 LR. Oysters had shelf life of 6-8 days at storage of 5°C, or 16-18 days when stored in ice.
Phuvasate 2015	HHP	All clinical strains completely inactivated to < LOD by 300 MPa for 5 min (>6,2 to >7.7 log reductions). Pressure resistance varies among strains. Reductions of 5 clinical *Vp* strains after 200-250 MPa at 20°C differed by up to 3.1 log CFU/ml.
Phuvasate 2015	HHP, low temperature	Decreasing temperature to 1.5°C sig. increased LRs of all clinical, environmental strains as compared to LRs at 5°C. HHP efficacy sig. enhanced at cold temps
Prapaiwong 2009	HHP, storage	HHP effective in reducing microbial loads in raw oysters, but large numbers bacteria survived treatment and proliferated during refrigeration.
Vu 2018	HHP	*Vp* in pure culture achieved 5 log reductions at 350 MPa for 1 min.
Ye 2011	HHP, incubation temp.	*Vp* cultures grown at 15°C were most sensitive to pressure, with no survivors detected (> 7 LR at 250 and 300 MPa. Differences in pressure resistance by incubation temp.
Ye 2012	HHP	To achieve a >3.52 LR of *Vp* in oysters by HHP alone, apply at least 275 MPa for 2 min
Ye 2012	HHP, mild heat	Conditions needed to achieve >3.52 log reduction: 200 MPa & 45 °C for 10 min, 200 MPa & 50 °C for 2 min, 250 MPa & 40 °C for 10 min, 250 MPa & 45 °C for 5 min, 300 Mpa, 50 °C for 5 min.
Ye 2013	Storage (varying temp.) then HHP	Cold storage at − 18, 4 and 10°C, prior to HHP decreased *Vp* by 1.5–3.0 log MPN/g but did not increase sensitivity to subsequent HHP.
Ye 2013	HHP then cold storage	Results for whole shell oysters are comparable to that of oyster meat. *Vp* populations slightly lower in whole-shell oysters than in oyster meat but differences not stat sig.

Table A15. Qualitative Findings: Icing.

Study	PHP Method	Qualitative Findings
Melody 2008a	On-board or dockside icing, cold storage	On-board and dockside icing did not predictably reduce levels of *Vp* in oysters; icing negatively impacted oyster survival during later cold storage.
Lydon 2015	Ice slurry	*Vp* load in water samples increased, but *Vp* load in oyster meat was unchanged after oysters submersed in ice slurries for 15 min. Oysters reached internal temp of 10°C in less than 12 mins.
Thomas 2016	On-board icing	Total *Vp* differed sig. across months, increasing in summer, but not sig. across treatment (iced) and control. Pathogenic *Vp* levels low and not sig. different across month and treatment. Data do not support efficacy of rapidly cooling oysters immediately after harvesting during warmer months. Sig. difference in mortality between control and treatments, iced oyster mortality must be $\leq$ 15% compared to controls for the process to be considered successful; that requirement not met by this process.

Table A16. Qualitative Findings: Irradiation, electric current.

Study	PHP Method	Qualitative Findings
Abdallah 2009	Gamma Irradiation	Varied response of tested *Vp* strains to g-irradiation based on virulence gene expression: g-irradiation can reduce or enhance virulence. After treatment, increase in *toxS* gene. mRNA quantities of *toxR* gene remained stable.
Andrews 2011	Irradiation	2.0 kGy dose required to reduce Vp from 7 log/g to non-detectable levels; 1.5 kGy to reach non-detectable levels from log 4/g.
Hou 2016	UVA Irradiation	UVA irradiation alone (3.81×10^4 J/m^2/min) was bactericidal.
Hu 2005	HHP, Gamma Irradiation	D for *Vp* in irradiated oyster tissues were lower than for *Vp* in pure culture.
Jin 2009	Low-amperage electric current	At 263 mA, *Vp* completely killed after 100 ms of treatment. At 526 mA all *Vp* killed regardless of treatment time. Interestingly, *Vp* can be selectively killed while leaving *Escherichia coli* and *Staphylococcus aureus* alive.
Mahmoud 2009	X-ray irradiation	> 6 LR achieved with .75, 2 and 5 kGy X-ray for pure culture, half shell and whole shell oysters respectively.
Nakahashi 2014	UV Irradiation	Survival ratios (log 10) after irradiation of UVA or UVC alone were half of survival ratio after sequential irradiation with LED. Bactericidal effect achieved with irradiation of UVA-LED or UVC alone.
Thupila 2011	Irradiation	Low-dose irradiation can reduce *Vp* populations without adversely affecting sensory characteristics. Lower D_{10} values in buffer than oyster meat.
Yagi 2007	UV-LED Irradiation	*Vp* was inactivated completely (100% inactivation rate) in 9 J/cm2. UV-LED can sterilize almost completely by irradiating with UV-Led for about 30 min.

Table A17. Qualitative Findings: Other PHPs.

Study	PHP Method	Qualitative Findings
Chen 2018	D-Tryptophan	D-Trp effect on growth inhibition depends on environmental NaCl concentrations, higher NaCl (>4%) and D-Trp(>20mM) has higher *Vp* growth inhibition. In artificial seawater it has potential to control *Vp* in live oysters at ambient temp and to extend shelf life of shucked oysters at refrigeration temp.
Fujikawa 2009		Developed a predictive program for *V. parahaemolyticus* growth based on O3:K6 strain growth data cultured at various broth temperatures, pH and salt concentrations. A logistic growth model with a lag phase was used to develop the program, and a 3-order polynomial equation analyzed growth from the data sets based on temperature, salinity, and pH.
Shi 2017	Low salinity stress, low salinity adaptation	*Vp* strains 1278 and ST550 were the most resistant to lethal low salinity, with ST550 surviving the lethal low salinity challenge. Exponential phase cells that underwent low salinity adaptation were sig. more resistant to lethal low salinity than non-adapted cells, wth 3 log CFU/ml greater survival.
Wang 2018b	Food matrix	Two pathogenic reference *Vp* strains (ATCC 17802, ATCC 33847) grew very slowly up to 3 hr, *Vp* abundance increased from 3 to 6 hr in oysters, indicating a 3 hr lag period. Little hemolytic activity for 3 hr; hemolytic activity in oyster reached stable phase after 12 hr, final hemolytic activity much lower. Linear fitted model plateaus and parallels the time axis. Time range for linear fit of hemolytic activities was 6 to 12 hr. Hemolysin could potentially remain active in contaminated food for up to 30 hr. Linear fitted model shows goodness of fit of hemolytic activity data with max growth rate period of *Vp* varies in different food matrices.

Table A18. Qualitative Findings: Relay.

Study	PHP Method	Qualitative Findings
Elmahdi 2018	Salinity relay	Salinity relaying did not sig. effect virulence genes or PFGE profiles but it did reduce *Vp* counts in oysters.
Jahncke 2011	High salinity relay	Salinity levels of 34 ppt tended to increase *Vp* numbers over 7 d. At 8 ppt, *Vp* numbers substantially decreased from start to final count on day 7.
Jahncke 2011	Storage, High salinity relay	Though initial numbers were higher after 7 h storage then for oysters immediately re-submerged, *Vp* counts decreased substantially by day 6 at 8 ppt and by day 12 at 17 ppt.
Melody 2008b	High salinity relay	Experiment had to end after 1 week due to equipment failures so there are only preliminary vibrio DNA probe results: results from days 0 to 7 indicate moving oysters from medium salinity (15 ppt) to higher salinity (30 ppt) can sig. reduce *Vp* counts. After 1 week relay at the site, *Vp* counts did not differ sig. from samples iced immediately after harvesting. However, 1 week of high-salinity relay did not reduce *Vp* to non-detect.
Parveen 2017	High salinity relay	Reductions in *Vp* were more consistent across replicates / composites at high salinity sites. Most reductions occurred and were stat. sig. after 14 and 21 d. Approx. 2 LR of *Vp* after 21 d.
Taylor 2018	High salinity relay	Results suggest that relaying oysters to reduce *Vp* levels holds promise, but both microbial community and environmental conditions (water temp, salinity) at relay sites influence efficacy.
Walton 2013	High salinity relay	*Vp* levels reduced to initial harvest levels within 14 d at 2 sites. Preliminary results indicate relaying oysters to relatively high-salinity waters shows some promise to reduce *Vp* abundance to initial harvest levels. However, population dynamics during first days is uncertain / not linear, *Vp* increased by day 2.
Yu 2010	High salinity relay	>1 log reduction in *Vp* concentrations, but reductions not sig. for natural relay. Relaying oysters to the creek is the most effective treatment among those tested.

Table A19. Qualitative Findings: Re-submersion.

Study	PHP Method	Qualitative Findings
Grodeska 2017	Desiccation, re-submersion	Levels of *Vp* were higher in oysters that underwent air-dry (desiccation) and freshwater dip treatments. In most cases there were not sig. differences between oysters that were air-dried vs. freshwater dipped. Time when *Vp* levels began to sig. decrease from before desiccation levels varied among trials. In most treatments, *Vp* levels returned to submersed (control) levels between 2 - 3 days post-submersion. In one trial *Vp* did not return to submersed levels until 3 to 7 days post-submersion, oysters had lowest overall average *Vibrio* levels at baseline. *Vp* levels in oyster that were air dried and freshwater dipped did not differ significantly 7 days post-re-submersion from *Vp* levels in continually submersed oysters (controls) Results of study support a minimum 7-day re-submersion regimen, (following a 27hr air drying or 3 hr freshwater dipping and 24 hr air drying).
Jones 2016	Intertidal exposure, Re-submersion	In WA state, mean *Vp* increased 1.38 log MPN/g after 4 hr intertidal exposure and decreased 1.41 log MPN after 1 day of re-immersion, levels dependent on container type. Pathogenic Vp followed similar trend. Overall data indicate intertidal harvest and handling practices do not increase risk of illness. Increase in internal oyster temperature following intertidal exposure corresponded with a stat sig increase in levels of total *Vp* and *trh*+, but not *tdh*+ *Vp*.
Kinsey 2015	Dry storage, Re-submersion	In most cases, *Vp* returned to levels similar to those measured at initial harvest, and concurrent background samples after 7 d re-submersion. Mean *tdh*+ levels were sig different from background levels after 7 d re-submersion. Mean *tdh*+ and *trh*+ levels after 14 d resubmersion were not sig different from background levels.

Table A20. Qualitative Findings: Temperature shock, stress.

Study	PHP Method	Qualitative Findings
Aagesen 2014	Heat shock	Heat shock sig. affects depuration efficacy in some but not all months.
Chang 2004	Heat shock	Differential response to heat shock across strains.
Chang 2004	Heat shock, heat stress	Regardless of heat shock treatment, heat shock increased thermal tolerance of strain 690 and chopping board isolate. For strain 690, significant increase in thermal tolerance with increasing duration of heat shock treatment.
Chiang 2008b	Heat shock	Heat shock increased TDH quantity in cell-free supernatant by 2-4 times depending on duration of treatment. Heat-shocked cells required more extended lag period to recovery than did control and ethanol-shocked cells.
Chiang 2009	Heat shock	% survival varies based on growth phase.
Chiang 2014	Heat stress	Survival of *Vp* to heat stress decreased with exposure period.
Hasegawa 2013	Freeze-thaw, heat shock	Heat tolerance of clinical strains *tdh*+ and/or *trh*+ was sig. higher than heat tolerance of strains *tdh*+ and/or *trh*+ from coastal environment and seafood. Heat tolerance of *tdh*- and *trh*- strains was significantly higher than heat stress tolerance in *tdh*+ and/or *trh*+ strains. No significant differences in tolerances against freeze-thaw and low osmolality stresses between *tdh*- and *trh*- strains and *tdh*+ and/or *trh*+ strains.
Kalburge 2014	Cold stress, high salinity pre-adaptation	Survival rate decreased as length of exposure time to cold stress increased. Cells preadapted in 3% NaCl had greater survival than cells preadapted in 6% NaCl.
Kalburge 2014	Heat stress, high salinity pre-adaptation	Survival rate decreased as length of exposure time to heat stress increased. Cells preadapted in 6% NaCl had greater survival than cells preadapted in 3% NaCl
Lai 2013	Cold stress, low salinity, starvation	Individual application of low salinity, low temperature or starvation for 24 hr did not sig. affect *Vp* survival. Combination of low salinity, temperature, or all three sig. reduced *Vp* survival.
Nishina 2004	Temperature stress, acid stress, salinity	*Vp* grew most rapidly at 25°C, pH of 7 or 8, in the presence of 1 or 3% NaCl. Both strains of serotype O3:K6 grew more rapidly than other strains in 1% and 3% NaCl at 15°C, at all pH levels. Data suggest serotype O3:K6 may survive better at low temperatures than other strains.
Whitaker 2010	Acid stress, cold stress	Cells grown at 3% NaCl are more resistant to high and low temperature stresses than cells grown at 1% NaCl.
Zarei 2014	Heat shock	Viability of both control and Cl-stressed, pathogenic and non-pathogenic cells decreased as incubation time at 50°C increased. No sig. difference across strains or treatments.

Table A21. Information to be extracted for the Dose-Response Model.

	Dose-Response Model
Dose-response relationship	Dosed with *Vp,* dose level reported Sample size (for quantitative data) Pathogenic strains (virulence factors, too, if available) used, reported Parameter estimates to inform uncertainty, variability Model type and equation Study strengths and weakness
Epidemiology adjustment	Estimates of annual illness Proportion of underreported to reported illnesses Proportion of under-diagnosed to diagnosed illnesses Parameter estimates to inform uncertainty, variability

Table A22. Information to be extracted for the Harvest Model.

	Harvest Model
Initial harvest levels of *Vp*	Water temperature Other environmental parameters Region of data collection
Intertidal growth equation	Air temperature Tissue temperature (can be >10°C higher than air on sunny day) Duration of intertidal exposure Method of intertidal harvest Substrate (mud vs rock)
Total Vp/g at harvest	Density of total, pathogenic and outbreak strains Pathogenic and total Vp in oysters, water

Table A23. Information to be extracted for the Consumption Model.

	Consumption Model
Consumption practices	Serving size (number oysters consumed per serving) Oyster weights Demographic characteristics (susceptible populations) Preparation practices used
Pathogenic Vp/g oyster at retail	Virulence factors, strains used

Supplemental File 1: Included Studies for the Dose-Response, Harvest, and Consumption Modules; studies excluded from the Post-Harvest Module during full-text review.

Supplemental File 2: Data Extraction File.

Curriculum Vitae

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EDUCATION

Johns Hopkins Bloomberg School of Public Health—Baltimore, MD
Expected Graduation: May 2019
•Master of Science Candidate in Environmental Health and Engineering (GPA: 3.84)
•Certificate in Risk Sciences & Public Policy
•Certificate in Quantitative Microbial Risk Assessment Interdisciplinary Instructional Institute
University of Maryland—College Park, MD Graduation: May 2017
•B.S. in Environmental Science & Technology, concentration in Environmental Health
•B.A. in Government & Politics
•Study Abroad, Center for International Educational Exchange in Costa Rica June-Aug 2015

HONORS & AWARDS

•JHSPH Centennial Scholar
• EHE Scholarship Award for Excellence in Academics & Environmental Health Achievement
•Travel Grant recipient for QMRA Interdisciplinary Instructional Institute
•JHSPH Student Assembly Student Conference Fund Recipient
•NOAA Hollings Scholar
•Maryland Senatorial Scholarship
•University of Maryland Dean's Scholarship

EXPERIENCE

Master of Science Candidate Research—Baltimore, MD Completion: May 2019
•Advisors: Dr. Keeve Nachman, Dr. Ben Davis
•Thesis project: quantitative microbial risk assessment (QMRA) on the risk of human infection and illness with *Vibrio parahaemolyticus* among shellfish consumers in Chesapeake Bay and Puget Sound. Conducted comprehensive literature review using methods similar to systematic review.
•Presented poster at World Aquaculture Society's 2019 Aquaculture Meeting in March 2019, titled Identification of post-harvest inputs for Vibrio parahaemolyticus Quantitative Microbial Risk Assessment in Chesapeake Bay and Washington State
•Presented poster at Delta Omega Poster Session in February 2019
•Presented poster at EHE Research Retreat in January 2019
JHSPH Dept. of Environmental Health & Engineering—Baltimore, MD Sept. 2017-present
Research Assistant for Dr. Meghan Frost Davis
•Perform environmental microbiological laboratory duties for multiple studies involving the microbial intersection of humans, animals, and environment; assist with study coordination; train and supervise interns.
•Investigated association of environmental exposures, risk factors with *Staphylococcus aureus* carriage among Baltimore city homes with children with persistent asthma. Earned course credit for Statistical Methods in Public Health IV.

Earthjustice—New York, New York October 2018-present
Extern
Evaluate comments submitted to Environmental Protection Agency scope and problem formulation documents in support of ongoing EPA risk assessments for ten chemicals under the Toxic Substances Control Act. Evaluate documents for legal compliance and reflection of the best available science.

Occupational & Environ. Epidemiology Branch, NCI—Shady Grove, MD June-Aug 2018
Summer Fellow for Dr. Mary Ward
•Obtained, cleaned and analyzed data to create exposure metrics that integrated drinking water contaminants to identify and understand exposure-disease associations in North Carolina participants of the Agricultural Health Study, contributing to development of environmental exposure assessments.
•Presented poster at DCEG Summer Fellows Poster Day in August 2018, titled Exposure assessment of drinking water contaminants among North Carolina participants in the Agricultural Health Study.

QMRA Interdisciplinary Instructional Institute—Columbus, Ohio July 2018
•Developed QMRA for Hepatitis A virus transmission among homeless populations in San Diego, California. Conducted exposure and dose-response assessment, model development with interdisciplinary team.

Maryland Department of Agriculture —College Park, MD May-August 2017
Field Technician
•Trapped, sorted and identified adult mosquitoes for infectious disease examination, applied larvacides to larval populated areas, performed Zika and West Nile Virus response, and educated residents about mosquito control.

National Oceanic and Atmospheric Administration—Silver Spring, MD May-August 2016
Hollings Scholar Intern
•Gathered data, performed literature review and Ecosystem Services Assessment for aquaculture nitrogen removal in Choptank Habitat Focus Area. Used Excel to analyze, prepare data for Assessment of Estuarine Trophic Status.
•Oral presentation at **Association for Sciences of Limnology and Oceanography** 2017 Aquatic Sciences Meeting in Honolulu, HI in March 2017.

UMD Dept. of Environmental Science & Technology—College Park, MD Jun 2014-May 2017
Research Assistant, Surveyor for Dr. Paul Leisnham
•Collected mosquito samples in Baltimore and ovitrap in Riverdale, MD. Identified mosquito species using microscope, ran differential scanning calorimeter for metabolic rates, maintained larval colonies, lab and greenhouse facility.
•Administered stormwater management/ BMP surveys to Eastern Shore farmers, to Maryland and D.C. residents.

Environmental Science & Technology Capstone—College Park, MD Sept 2016-May 2017
•Worked with team to design, perform assessment of chronic sediment and water toxicity and sediment microplastics at Anacostia River sites. Conducted literature review. Ran short-term static renewal toxicity tests.

Center for International Educational Exchange—Monteverde, Costa Rica June-Aug 2015
•Fieldwork and independent research project in tropical ecology. Performed altitudinal transects of epiphyll abundance on *Geonoma spp.* in the Monteverde montane cloud forest, replicating earlier study. Conducted statistical analysis. Presented poster at **National Center for Undergraduate Research** in Asheville, NC April 2016.

Teaching Experience:
Johns Hopkins Bloomberg School of Public Health
•*Teaching Assistant* for Methods in Quantitative Risk Assessment August-October 2018
•*Teaching Assistant* for Introduction to the Risk Sciences and Public Policy Aug-October 2018
•*Teaching Assistant* for Risk Policy, Management and Communication Oct- December 2018
•*Teaching Assistant for* Topics in Risk Assessment March-May 2019

Publication: M. Spaur. 2016, Palm Epiphyll Cover Shifts to Higher Elevations in Tropical Cloud Forest, Indicating Local Climate Change, *NCHC Journal of Undergraduate Research & Creative Activity*, On-Line Publication. Retrieved from http://www.nchc-ureca.com/assets/ureca_volume1-2.pdf

Miscellaneous Publication:
M. Spaur. 2018, We need to respond to climate change immediately, Washington Post, Opinion, Letters to the Editor. https://www.washingtonpost.com/opinions/we-need-to-respond-to-climate-changeimmediately/2018/09/03/a119fb8cad52-11e8-9a7d-cd30504ff902_story.html?outputType=comment&tid=sm_talk&utm_term=.877961d5dafa

PROFESSIONAL/ VOLUNTEER AFFILIATIONS
Johns Hopkins Bloomberg School of Public Health
•Johns Hopkins Science Policy Group, *President* September 2018-present
•Johns Hopkins Science Policy Group, *Project Lead, Executive Board Member* Oct 2017-2018
•Environmental Health & Engineering Student Organization, *member* Sept 2017-present
University of Maryland
•University Senate Executive Committee, *Undergraduate Representative* Sept 2016-May 2017
•Student Government Association Sustainability Committee, *Director* Aug 2015-Dec 2016
•Sustainability Fund Review Committee, *member* September 2015-May 2017
•Sustainability Advisor September 2015-May 2017
Miscellaneous
•Climate Reality Leader March 2019
SKILLS
•Microsoft Office Suite: Excel, Word, PowerPoint, Publisher
•Crystal Ball •STATA, SAS, SPSS •ArcGIS
•Public Speaking •Speak, read and write in Spanish (intermediate)
•Mosquito identification